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IMMUNOLOGICAL STUDIES IN PNEUMONIA.

By RICHARD WEIL, M.D., and JOHN C. TORREY, Ph.D.

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(Received for publication, July 20, 1915.)

The immunological processes of pneumonia have been studied within recent years by many observers, among whom may be mentioned Klemperer,[1] Römer,[2] Neufeld and Haendel,[3] Wadsworth,[4] Dochez,[5] and Chickering.[6] It has been established that a certain type of immune body, namely, the agglutinins, tends to appear in the blood shortly after recovery from the disease, and to persist therein for a variable period of time. During the course of the disease this antibody has only exceptionally been found. In addition, specific protective substances have been found in convalescent serum.

During the past winter we have approached the problem with the help of a method not previously employed in the study of this disease. Essentially, the method consists in passively sensitizing guinea pigs with serum taken from cases of pneumonia at various stages of the disease, and during convalescence. Passive sensitization of guinea pigs with human serum had previously been practised by Bruck[7] and Schloss in the study of food idiosyncrasies. The application of the method to the study of infectious diseases has been attempted in the case of tuberculosis.

[1] Klemperer, G., and Klemperer, F., *Berl. klin. Wchnschr.*, 1891, xxviii, 833.

[2] Römer, Experimentelle und klinische Grundlage für die Serumtherapie der Pneumokokkeninfection der menschlichen Cornea (Ulcus serpens), Wiesbaden, 1909.

[3] Neufeld, F., and Haendel, *Arb. a. d. k. Gsndhtsamte*, 1910, xxxiv, 166, 293.

[4] Wadsworth, A., *Am. Jour. Med. Sc.*, 1904, N. S., cxxvii, 851; *Jour. Exper. Med.*, 1912, xvi, 54.

[5] Dochez, A. R., *Jour. Exper. Med.*, 1912, xvi, 665.

[6] Chickering, H. T., *Jour. Exper. Med.*, 1914, xx, 599.

[7] Bruck, C., *Arch. f. Dermat. u. Syph.*, 1909, xcvi, 241.

One of the most serious difficulties associated with this method lies in the fact that human serum is extremely toxic to guinea pigs. After a considerable amount of preliminary investigation we finally adopted the use of inactivated serum, as recommended by Schloss[8] and others. For the sake of uniformity we have, in all except the first studies, regularly injected 4 cc. of inactivated serum into the subcutaneous tissues. After a period of time varying from two to six days, the guinea pigs have been tested to determine whether or not they presented an induced hypersusceptibility to the pneumococcus. The method of performing this test differs from that hitherto observed in this type of experiment. The routine test, as usually performed, consists in the introduction of the antigen either into the peritoneum or into the veins of the sensitized animal. In the study herein described that method was not practicable.

The antigen was prepared in the following manner. Forty-eight hour cultures on Loeffler's serum medium (ox serum) were emulsified in distilled water, using 2 to 3 cc. of water for each tube, according to its size. The emulsion was shaken and autolyzed at 37° C. for two hours, and then at 60° C. for one hour.[9] With a very few exceptions, the organism employed was of Type I. In a few of the earlier experiments a Group III type (*Pneumococcus mucosus*) was used with success. It seems probable that this anaphylactic reaction is more general for the whole pneumococcus family than has been found to be the case in agglutination and protective experiments. This autolytic extract when injected intravenously into guinea pigs is primarily toxic, but the toxicity varies with different animals to a considerable extent, so that the reaction produced in the anaphylactic experiment is unreliable and confusing. As an alternative, the induced hypersusceptibility of the guinea pigs was tested by the Dale[10] method. This method consists in removing the uterus of the sensitized animal and suspending it in a bath of Locke's fluid. The uterine preparation traces a rhythmical curve, which changes strikingly upon the addition of antigen to the bath fluid. There is an immediate, or slightly delayed, response in the nature of a sharp

[8] Schloss, O. S., *Am. Jour. Dis. Child.*, 1912, iii, 341.

[9] Dochez, A. R., and Gillespie, L. J., *Jour. Am. Med. Assn.*, 1913, lxi, 727.

[10] Dale, H. H., *Jour. Pharmacol. and Exper. Therap.*, 1912–13, iv, 167.

contraction, which may be followed either by prompt relaxation or by tetanus of the muscle. In testing the pneumococcus autolysate, it is necessary to keep in mind certain important factors. In the first place, the antigen of itself possesses in a certain degree the property of stimulating a contraction, even when added to the normal uterus. In this respect, however, it does not differ from normal sera, such as horse serum or ox serum, which have the same effect upon the normal guinea pig uterus. An essential feature of the method therefore consists in the use of the antigen in such amounts as do not, of themselves, suffice to induce contractions in the control uterus.

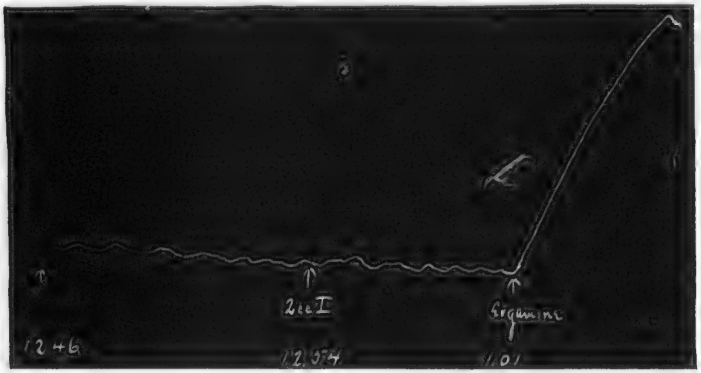
In the present study we have made a practice of using approximately one-quarter of that amount which just fails to induce a response in the normal uterus. As a rule, 1 cc. of extract, which has always been uniformly prepared throughout the series, is added to a bath containing 150 or 200 cc. of fluid. It is a necessary precaution to test each sample of autolysate, in order to be sure that its primary toxicity is not greater than is assumed for this amount. Occasionally it has been found necessary to diminish the amount of antigen used in the tests on account of an unexpectedly high primary toxicity. The other technical details of the method are not such as to require special discussion in this connection.

OBSERVATIONS.

Blood has been taken and tested from a series of cases which presented themselves, clinically, as pneumonias, as well as from a series of controls which were either normal individuals or cases of other forms of disease; such as typhoid, rheumatism, etc. In every case of pneumonia the sputum was cultured in order to determine the etiological organism, and in some cases blood cultures as well were made; in all, 20 cases of pneumococcus, 2 of *Streptococcus mucosus*, 2 of streptococcus, and 1 case of *Bacillus mucosus* were determined. In 9 cases the organism was not definitely determined.[11]

[11] The sputum examinations and the blood cultures were carried out for the Mt. Sinai cases by Dr. H. Celler, to whom we wish to express our indebtedness. He reported on sputa only when the plates presented practically pure cultures of the organism.

Leaving out of consideration the cases of questionable etiology, none of those cases which were bacteriologically identified as other than pneumococcus infections, with the exception of one case of *Streptococcus mucosus* infection, gave a positive response. Of the twenty cases of pneumococcus infection, there were only two which did not at some stage of their course present a positive response. Of the questionable cases, in which the bacteriological diagnosis could not be obtained, there were four in which the clinical pictures seemed, with certainty, to exclude the possibility of a pneumococcus infection. In none of these four was a positive result obtained. In



TEXT-FIG. 1. Guinea pig 583. Received 4 cc. of inactivated serum derived from a case of acute articular rheumatism. Tracing, taken on fourth day, shows no reaction to pneumococcus antigen, although there is a sharp response to ergamine.

the other cases of that group it was not possible to decide, with any degree of certainty, whether or not the infection was due to pneumococcus. The results obtained in these cases were in some instances positive, and in others negative.

The time of occurrence of reaction differed from that observed by others in the study of the agglutinins. In all of the cases in which it occurred, it appeared before the temperature had reached normal. In only two of these cases was it also present during convalescence, and then in far less marked degree. The reaction was present within

two days of the onset of the disease in one case, within three and four days in certain other cases. When found, it persisted almost unexceptionally up to the time of crisis; or if the disease ended by lysis, it persisted throughout the lysis. Two of the cases ended fatally; in one of these it was found throughout the disease; in the other, which died upon the day of admission, it was not found. Those two cases in which it persisted after crisis did not appear to differ according to any clinical criteria from those in which it disappeared. In none of the control cases was the reaction induced.

The conditions which have been described may fitly be illustrated by a number of typical tracings. Text-fig. 1 shows a tracing taken from

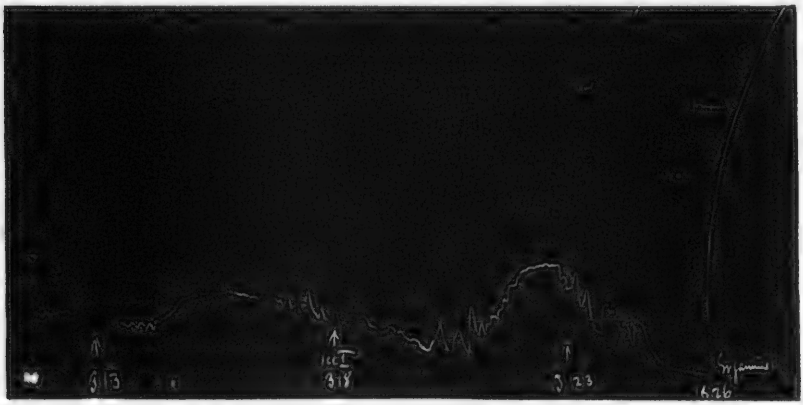
TEXT-FIG. 2.

TEXT-FIG. 3.

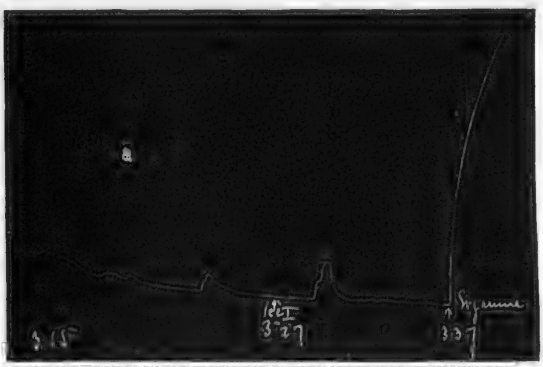
TEXT-FIGS. 2 AND 3. Guinea pig 565. Received 4 cc. of inactivated serum derived from a case of acute lobar pneumonia due to pneumococcus, five days after onset. Tracings, made on fifth day, show a sharp response to antigen.

the uterus of a guinea pig which had received 4 cc. of the inactivated serum of a patient suffering from an attack of acute articular rheumatism. The temperature at the time the blood was taken was 103° F. It is evident that there is no response to the application of the pneumococcus antigen. Text-figs. 2 to 7 illustrate the course of a case of lobar pneumonia. The patient was admitted to Mt. Sinai Hospital on the fourth day of the disease. The onset had been acute and was marked by a chill. Upon admission, there were characteristic signs of consolidation of the middle and lower lobes of the right

lung. The temperature was 105.4° F., and the leucocyte count 15,200, of which 85 per cent were polynuclears. The temperature varied only slightly for four days, and on the fifth day fell by uninterrupted crisis. Recovery was uneventful from the crisis. The clinical picture was that of a lobar pneumonia of moderate severity.



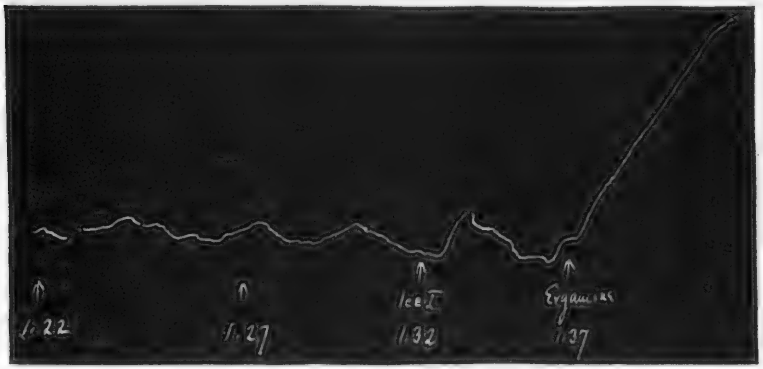
TEXT-FIG. 4.



TEXT-FIG. 5.

TEXT-FIGS. 4 AND 5. Guinea pig 572. Received 4 cc. of inactivated serum derived from same case during crisis. Tracings made on third day. There is no response to antigen. Ergamine produces a sharp contraction.

Pneumococci were found in the sputum, but not in the blood. Blood was taken for the anaphylactic tests on three occasions; namely, during the height of the disease, during the crisis, and on the third day after the crisis. On each occasion the blood was subjected to the same procedure. The serum was allowed to separate from the clot,



TEXT-FIG. 6.

TEXT-FIG. 7.

TEXT-FIGS. 6 AND 7. Guinea pig 588. Received 4 cc. of inactivated serum derived from the same case three days after crisis. Tracing taken on fourth day. One horn shows a minimal response to the antigen. Both horns respond well to ergamine.

and was then centrifuged to free it of cells. It was then inactivated for one-half hour at 56° C. Of this serum, 4 cc. were injected subcutaneously into a female guinea pig weighing between 250 and 300 gm. After an interval of from three to five days the guinea pig was killed, and the uterine horns were immediately removed and suspended, according to Dale's method, in a bath containing 200 cc. of Locke's fluid. The reaction to the pneumococcus autolysate was tested, and if this was negative, ergamine was subsequently added to establish the normal reactivity of the muscle. The figures show that the blood taken during the course of the disease had effectively sensitized the uterus to the pneumococcus antigen. The blood taken during the crisis and after the crisis failed to induce this result, although the muscle evinced a normal reactivity towards ergamine.

Of interest in connection with our own results are those described by Seligmann.[12] This author reports that he attempted passive sensitization of guinea pigs with postcritical and precritical serum, but that he failed to achieve any positive result. He gives no detailed protocols of his experiments, but describes his method, which differs in practically every detail from that employed in the present study. He sensitized by means of the intraperitoneal injection of 5 to 6 cc. of the human serum. We have almost regularly found this dosage fatal for the guinea pig, and therefore inactivated the serum and gave it subcutaneously. The interval between passive sensitization and the toxic injection in Seligmann's experiments was only one day, whereas we allowed three or more days for the absorption of the human antibodies. His second injection consisted of a suspension of a pneumococcus culture, while we used the soluble autolysate. Finally, he employed the intravenous injection, whereas we tested by the Dale method. Without further analysis, it must be clear that any one of these differences in technique would suffice to account for the divergence in results.

DISCUSSION.

The question which immediately arises naturally involves the nature of the substances in the sera concerned in the production of

[12] Seligmann, E., *Ztschr. f. Immunitätsforsch.*, 1911, ix, 79.

this reaction. There is practically no doubt that they must be grouped among the immune substances, the so called antibodies. There are no other substances, so far as we know, which passively sensitize a guinea pig's uterus. Apparently they are specific in character. Whether or not they have protective value is a problem which has not been approached. The fact that their incidence in the disease differs strikingly from that of the agglutinins and the lytic antibodies, suggests the likelihood that they may be different from those substances. In fact, there is some ground for the belief that hemolysins, agglutinins, and precipitins may not be identical with anaphylactic antibodies, judging from the fact that the first group of substances does not always run parallel with the second in amount.

Such a conclusion, however, does not necessarily follow. It is possible that exactly the same substance acts as agglutinin in the test-tube and as sensitizing substance in the guinea pig, and that the apparent difference in the effects observed by the two methods is due simply to the coexistence of varying amounts of antigen. According to this view, agglutination is inhibited in the precritical serum through the presence of antigen, a factor which does not, however, inhibit passive sensitization. The postcritical serum, on the other hand, might contain less antibody, so as to impair its sensitizing value, but might at the same time be almost free from antigen, so as to permit of the demonstration of agglutinins in the test-tube.[13]

The clinical material on which this work is based was obtained chiefly from the service of Dr. Libman at the Mt. Sinai Hospital, and in part also from the service of Dr. Brill at the Mt. Sinai Hospital, and from the service of Dr. Coleman in Bellevue Hospital, for whose cooperation we desire to express our thanks. We are also indebted to Drs. Cole and Dochez of The Rockefeller Institute Hospital for pneumococcus cultures and sera.

[13] The experiments on which this theory is based are included in a series of papers by Weil, R., *Jour. Immunol.*, 1916, i (in press).

CONCLUSIONS.

The serum derived from human cases of pneumonia has been used to sensitize guinea pigs passively. As antigen an autolysate of a pneumococcus culture was employed. By means of Dale's method it has been possible to show with considerable regularity that the blood contains sensitizing antibodies during the course of pneumonia, but none after the crisis. Patients suffering from other diseases have failed to give this reaction, as have also normal individuals.

NOTE ON A SKIN REACTION IN PNEUMONIA.

By RICHARD WEIL, M.D.

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The present paper describes the results observed when pneumococcus antigen, prepared in the same way as for the anaphylactic experiments described in the preceding article,[1] was injected intracutaneously into a series of pneumonia patients, and into controls. The same method has been employed by Clough,[2] who reached the conclusion that these injections had in general an irritant effect, and that no sharp differences could be shown to exist between pneumonia cases and controls. The present observations do not coincide with those of Clough, but it seems likely that the divergence in results may possibly be due to a difference in the composition of the antigen employed. A very strong pneumococcus extract possesses primarily irritative properties, and sets up reactions independently of any immunological response. In Clough's work the dried and ground residue of a twenty-four to thirty-six hour culture was extracted at 37° C. for eighteen hours. The two hour autolysate employed in the present study is very much weaker than Clough's extract. The method of injection was intracutaneous, and the site chosen was the interscapular region. The autolysate, prepared as described in the preceding paper by Torrey and Weil, was kept in the ice box, and was never used more than one week after its preparation. During this period no change could be detected in its effects when injected into the skin. Not infrequently it happens that a sediment forms in the tubes containing the autolysate; this was not disturbed, but the supernatant fluid was carefully drawn off for the tests. As a rule, the autolysate has a faint whitish, opalescent appearance. The amount injected varied from 0.1 to 0.2 cc., depending on the texture of the

[1] Weil, R., and Torrey, J. C., *Jour. Exper. Med.*, 1916, xxiii, 1.

[2] Clough, P. W., *Bull. Johns Hopkins Hosp.*, 1915, xxvi, 37.

skin. The object in giving the injection was to produce a small wheal of the skin. The injection is immediately followed by a superficial, ill defined, cutaneous blush. This is present in pneumonia cases as well as in controls. It is believed to be due to a local irritant effect, and is not considered as a true reaction. The subsequent course of events at the site of injection varies under different circumstances. The superficial erythema may fade within a few hours, and nothing further may develop. Such a course is denominated as an absence of reaction, and the case is called negative. On the other hand, within the twenty hours following the injection further changes may occur at the site of injection. A fairly well circumscribed area of erythema, with slight infiltration and elevation of the skin surrounding the point of puncture, may develop. If the infiltration is marked, a true papule results. These changes may persist for forty-eight hours or more. They are described as a reaction, and a case presenting this is called positive. No attempt has been made in the following analysis to differentiate between the grades of positive reaction, or to distinguish the significance of an indurative from a papular reaction.

During the course of the disease the skin presents no reaction to the injection. After the subsidence of the disease, a considerable percentage of the cases do present a reaction. This reaction may exceptionally be induced within twenty-four hours after the crisis, or may make its appearance only after an interval of two or three or more weeks. The normal individuals, or the diseased controls, may or may not present a reaction, depending presumably on their previous sensitization by the pneumococcus or an allied organism. Such sensitization might presumably depend upon a previous mild and unidentified attack of the disease, or upon reaction to the presence of parasitic organisms in the throat. A few cases of pneumonia have been observed in which a reaction was not only absent throughout the disease, but failed to manifest itself at any time during the after period.

DISCUSSION.

The interpretation of these results is not entirely clear, owing to the fact that the mechanism of the skin reaction has not been sufficiently elucidated. Unquestionably, there are two types of skin reaction, of which the one, exemplified by the method of Römer and Schick in the case of diphtheria toxin, depends upon the primary toxicity of the antigen. A positive response in this type indicates a deficiency in the mechanism of defense. Probably Clough's reactions fall into this category. On the other hand, the antigen may have no primary toxicity, as, for example, horse serum or tuberculin, and in this case a positive response indicates the presence of antibodies. The observations herein described were based on the use of a nontoxic concentration of the pneumococcus autolysate. In the second place, it is not known whether the reaction is mediated by the circulating or by the fixed antibodies, and in either case to what extent it is modified by the coexistence of either fixed or circulating antigen. On the basis of animal experiments, the latter factor might be assumed to play a very essential part. Finally, the human being is a very unfavorable subject for the elucidation of skin reactions, presenting, in this respect, marked individual variations. Tentatively, we should be inclined to assume that the absence of a reaction during the course of the disease was attributable not to the absence of antibody, but to the coexistence of sufficient amounts of antigen within the cells to inhibit the reaction. With the diminution of antigen, which goes on progressively from the time of crisis, antibody may become available for a reaction. If the latter is present in sufficient amounts, the cutaneous reaction to the injection of antigen is pronounced; if not, it is absent. The absence of the reaction during the disease presents an interesting analogy with the effect of an acute general miliary tuberculosis on the von Pirquet reaction.

The work of Major and Morse[3] in many respects closely parallels the observations above recorded. A moderately severe type of reaction, described as erythemo-indurative, was observed in a large number of control cases. Cases of pneumonia, bacteriologically

[3] This work has not been published. The manuscript report was kindly sent me by Dr. Hirschfelder, under whose direction it was carried on.

identified only by microscopic study of the sputum, reacted regularly shortly after the crisis, but never during the disease. The reaction usually became papular in type. Except for the fact that the pneumonia cases invariably responded shortly after the crisis, these observations agree in essential particulars with the present series. In view of the fact that Major and Morse studied only four cases subsequent to the crisis, it seems not unlikely that the regularity observed by them might have disappeared in a larger series.

From a diagnostic standpoint, the reaction has no significance. The fact that it is absent during a pneumonic process does not sufficiently establish the bacterial etiology of the disease.

AN EXPERIMENTAL STUDY OF THE EFFECT OF CHOLECYSTGASTROSTOMY ON GASTRIC ACIDITY.

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INTRODUCTION.

The beneficial effects of gastro-enterostomy on ulcer of the stomach and duodenum are variously explained, some authorities maintaining that they result from a more complete and rapid evacuation of the stomach, others that the effect is due mainly to chemical changes in the gastric contents which result in a reduction of the total acidity. Between these two hypotheses there is still another view which attributes the improvement to a combination of both factors. W. J. Mayo, for example, believes that gastrojejunostomy not only affords better drainage but also alters the physiology of the stomach in a favorable way. In a similar manner Hartmann and Lecene, Noetzel, Collinson, and others hold that a partial neutralization of the gastric acidity results from the reflux of jejunal contents into the stomach. Finney and Friedenwald, who have recently drawn attention to the advantages of pyloroplasty over gastro-enterostomy, report a reduction of hyperacidity as one of the favorable results from this operation.

The secretions held responsible for this chemical change are bile, the pancreatic juice, and the juice from the duodenal mucosa. It has long been recognized that varying amounts of the duodenal contents are normally present in the stomach following operations of this nature; thus Kausch, Carle and Fantino, Kaiser, Hartmann and Lecene, and numerous others have regularly observed bile in the gastric contents in these cases.

If it be true that the regurgitated duodenal or jejunal secretions exert a favorable physiological influence on the gastric contents it is likely that this change consists of a reduction of the total acidity.

Such a change might be due to either one of two factors, or to a combination of them. First, any one or more of the secretions normally present in the duodenum on reaching the stomach might lead to a reflex decrease in the production of gastric juice. Second, since the duodenal contents are alkaline, any one or more of these secretions might neutralize the acid chyme of the stomach.

The purpose of the investigations reported here has been to determine if possible whether bile when it is introduced into the stomach plays a significant part in reducing the acidity of the gastric contents. The importance of the pancreatic and duodenal secretions in this respect have been left for further studies.

HISTORICAL.

The effect of the bile salts on gastric secretion has been studied clinically by Glaessner. After feeding sodium choleate to patients with hyperchlorhydria he noted a definite decrease in the acidity of the stomach contents.

Palfrey reports favorable results from feeding salol-coated pills of ox bile to patients with hyperacidity. He attributes this improvement to a neutralization by bile of the acid stomach contents when they reach the duodenum. That both human and bovine bile stimulate the secretion of bile has been shown by Pfaff and Balch.

Boldyreff showed experimentally that regurgitations of pancreatic juice and bile occur regularly after the ingestion of much fat, and in the presence of a large amount of free acid—hydrochloric and fatty—in the stomach, and suggests that the backward flow of alkaline duodenal contents may represent an attempt on the part of the body to neutralize the unusual acidity of the stomach.

Opposed to the findings of Glaessner are the results from certain experiments performed by Lönnqvist. He fed dogs prepared with Pawlow pouches with bile obtained from other animals having biliary fistulæ and found that it stimulated the secretion of gastric juice to the same degree as water; that is, the acidity and the digestive power of the juice collected after the ingestion of bile corresponded closely to the acidity and the digestive power of that obtained after the injection of water. The contents of the main stomach, however, subsequent to the ingestion of bile showed almost no digestive power, due to the precipitation of the pepsin by the bile.

Wiedemann anastomosed the gall bladder to the stomach in a dog and studied the gastric contents by means of a gastric fistula. While single examinations occasionally showed a slight decrease in the total acidity, others revealed an occasional increase. He concludes that the acidity of the gastric contents, at least when milk and eggs are fed, is not appreciably affected by diverting the bile into the stomach.

The effect of bile on the gastric mucosa has been studied by a number of workers. Rywosch concluded from feeding bile to chickens and rabbits that the introduction of large amounts might be very injurious to the stomach, whereas small amounts probably produce no ill effects. In the higher animals there is considerable evidence to show that bile exerts no injurious influence on the normal mucosa. Oddi, Dastre, and Smith diverted the bile from the duodenum into the stomach in dogs and noted no pathological changes in the gastric wall. Oddi also fed large amounts of ox bile to dogs for many days with similar results. In their cholecystgastrostomy experiments Dastre and Wiedemann analyzed the gastric contents and found digestion unaffected by the bile. Smith emphasizes the importance of mucus as a means of protecting the stomach against the action of chemical agents. While the introduction of weak hydrochloric acid and bile separately never led to lesions of the mucosa, combinations of the two, when injected into the stomach during the course of digestion (mucus being probably less abundant at this time), usually resulted in some necrosis of the epithelium.

Cannac, Masse, Mocquot, and others have reported an absence of ill effects following the anastomosis of the gall bladder to the stomach in dogs. Clinical experience has afforded similar results in man. Kehr, Jaboulay, Eichmeyer, Jacobson, and numerous other surgeons have found this operation in many instances to be preferable to cholecystenterostomy.

Methods Employed.

While Chigin has shown that the course of secretion in the smaller Pawlow stomach exactly corresponds to that in the larger, it was thought that by following the gastric secretion in both stomachs before and after the administration of bile each set of experiments would serve as a check on the other. Accordingly the work was divided into two parts. In one series of animals pure gastric juice was obtained from Pawlow pouches. So far as can be ascertained this method has not been used for investigating the gastric acidity in the presence of a cholecystgastrostomy. In the other series the gastric contents were obtained by injecting apomorphin hypodermically, as suggested by Hamburger and Friedman in their work on pyloric stenosis. Analyses were made throughout the various stages of digestion by repeating the test meal on different days, the stomach being emptied each day at a different interval after the ingestion of the food. An interval of one or more free days was always allowed between such analyses.

Great care was taken in forming the miniature Pawlow stomachs to prepare with a broad base the flap of the stomach wall used for

the pouch so that the pedicle of muscularis and peritoneum might carry an ample nerve supply to the pouch. It was found that the erosion of the abdominal wall about the pouch opening could be greatly minimized by leaving a full cuff of mucosa projecting above the surface of the abdomen. From a series of more than a dozen dogs prepared in this manner three were found to be satisfactory in every way for the investigation.

Bile was supplied by ligating and dividing the common duct and anastomosing the gall bladder with the stomach. The normal color of the stools, the absence of jaundice, and the patency of the anastomosis at autopsy proved that all of the bile had entered the alimentary tract via the stomach.

The operations were conducted under full ether anesthesia. In the animals reported here the wounds all healed by primary intention.

The analyses were made with dimethylamidoazobenzol and phenolphthalein. In the tables the results are expressed in terms of cc. of N/10 sodium hydroxide—acidity per cent. Ten minutes after the ingestion of the test meal the dog was placed in its canvas hammock and a collection of gastric juice was started. Analyses were carried out at fifteen minute intervals.

It is hardly necessary to add that an ample period of time was allowed after each of the operations in order that the animals might completely recover before the secretory activity of the stomachs was investigated.

The Effect of Cholecystgastrostomy on Gastric Acidity.

The Quantity and the Acidity of the Gastric Juice.—Table I is representative of the results obtained from following the gastric secretion by means of Pawlow pouches. Numerous examinations were made both before (controls) and after the diversion of the bile stream into the stomach. While slight variations from the controls were noted at times after the cholecystgastrostomies the minor degrees of hypoacidity were fully counterbalanced by slight degrees of hyperacidity. As a whole this method yielded very constant results.

The figures in Experiments IV and XIII are from the protocol of Dog II. Those in Experiment XIII were obtained four weeks sub-

sequent to the ligation and division of the common bile duct and the anastomosis of the gall bladder to the stomach. On comparing the two tables it is evident that there was very little difference in the amounts of gastric juice secreted from the small stomach before and after the operation. During the last hour and a half of the collec-

TABLE I.

Dog II.

Experiment IV.* Before cholecystgastrostomy.					Experiment XIII.* After cholecystgastrostomy.				
Time.	Juice.	Free acidity.	Combined acidity.	Total acidity.	Time.	Juice.	Free acidity.	Combined acidity.	Total acidity.
	cc.					*cc.*			
7.45	5.5	110	10	120	7.15	7.0	105	10	115
8.00	10.0	140	5	145	7.30	9.0	135	5	140
8.15	6.0	145	5	150	7.45	10.5	150	5	155
8.30	6.0	140	5	145	8.00	6.5	145	5	150
8.45	5.0	135	10	145	8.15	4.7	140	5	145
9.00	3.2	130	10	140	8.30	3.3	125	10	135
9.15	4.0	130	10	140	8.45	3.1	130	5	135
9.30	4.0	125	10	135	9.00	3.0	120	10	130
9.45	3.0	110	10	120	9.15	3.0	115	10	125
10.00	2.6	125	5	130	9.30	2.8	115	10	125
10.15	3.0	120	10	130	9.45	2.5	120	10	130
10.30	3.1	120	5	125	10.00	2.6	115	5	120
10.45	2.7	120	10	130	10.15	2.5	105	10	115
11.00 / 11.15	3.4	115	10	125	10.30	2.2	100	10	110
					10.45	2.0	95	10	105
11.30 / 11.45	2.7	115	5	120	11.00 / 11.15	3.1	95	5	100
12.00 / 12.15	1.0	110	5	115	11.30 / 11.45	2.0	90	10	100

* Test meal: chopped meat 150 gm. and water 140 cc.

tions the total acidity was somewhat lower in Experiment XIII. This feature, however, was not constant throughout the experiments, but was found to vary with the period of convalescence. Where the curve of secretion was investigated soon after the cholecystgastrostomy (three to four weeks), figures were obtained such as are recorded

here. Where analyses were made at later periods, on the other hand, the acidity values rose to a higher level. Two and one-half months subsequent to the operation no appreciable difference was noted in the figures obtained during the last hour and a half of the collections when these were compared with those secured previous to the gall bladder anastomosis. At this later date, nevertheless, during the first three hours of digestion a very slight rise in the total acidity was frequently found.

These results indicate then that bile when present in the stomach in considerable amounts during digestion exerts no appreciable influence on the secretion of gastric juice, and has no significance in decreasing the acidity of the gastric contents, at least with a diet of meat and water. While it is problematical just what quantity of bile reaches the stomach during this phase of digestion in such animals, it appears likely that the acid chyme entering the duodenum serves to activate the prosecretin as usual, and in this way stimulates a more or less normal secretion (Howell).

Foster and Lambert have called attention to the slight individual variations noted among dogs fed on the same kind of food, both in the amounts of secretion and in the percentage content of acid. They conclude that the only evidence of limitation of function that we can gain must depend, to be of value, upon an average computed from the data for a number of animals. In the experiments reported here this feature has no essential bearing on the results since comparisons of the data from one animal were not made with those from another. In each dog an average of the findings obtained during a number of tests before cholecystgastrostomy was contrasted with that obtained from a similar series of experiments conducted subsequent to the operation. As a matter of fact the data from the three animals showed very slight differences.

Each of the three dogs reacted well to the cholecystgastrostomy. There was no appreciable vomiting at any time and in each instance on the day following the operation the animal drank its allowance of broth. During the course of the experiments the stools remained colored and of normal consistency. The general nutrition of the animals continued good throughout the following months. In one instance the observations extended over six months.

The Acidity of the Gastric Contents.—The figures in Table II are from the protocol of Dog VI. They illustrate the average results obtained from following the acidity of the gastric contents before and after the diversion of the bile stream into the stomach. Here again subsequent to the cholecystgastrostomy variations from the controls were noted, both decreases and increases in the total acidity. These counterbalanced one another well, however, showing no appreciable average deviation from the normal. As a whole, the fluctuations in acidity were somewhat greater with this method.

TABLE II.

Dog VI.

Experiment II.*				Experiment V.*			
Before cholecystgastrostomy.				After cholecystgastrostomy.			
Hours after feeding.	Free acidity.	Combined acidity.	Total acidity.	Hours after feeding.	Free acidity.	Combined acidity.	Total acidity.
1	0	56	56	1	0	65	65
2	0	49	49	2	8	62	70
3	18	50	68	3	21	68	89
4¼	25	46	71	4¼	25	40	65
5	21	69	90	5	16	87	103
6	32	42	74	6	31	41	72
7	20	38	58	7	17	50	67
8½	15	30	45	8½	9	29	38

* Test meal: chopped meat 100 gm. and water 100 cc.

Experiment II presents the data secured about three months subsequent to the anastomosis of the gall bladder with the stomach. At this postoperative period the analyses showed a slightly increased acidity of the gastric contents as compared with the data obtained previous to the cholecystgastrostomy. The same inclination toward a hypersecretion was noted in the other two animals at corresponding periods. The results were a little different, however, when the analyses were made at shorter intervals after the operations. At the end of four or five weeks the acid values averaged slightly lower than those secured with the stomach in its normal state. The acidity of the gastric contents then increased somewhat as the weeks passed.

In no dog of the series was any marked or permanent decrease in the acidity found.

As a rule the test meals secured after the cholecystgastrostomies closely resembled macroscopically those obtained previous to the operations, except for a slight yellowish tint of the fluid portions. Occasionally a trace of free bile was evident.

Two of the dogs showed some temporary effects from the anastomoses. There was urgent emesis during the first twenty-four hours, and an occasional emptying of the stomach in the course of the next few days. With this was coupled a striking anorexia. As a result the nutrition of both animals was decidedly affected. During the second week, however, as the appetite improved each dog put on weight and became more active. The third dog manifested no ill effects of any kind. Throughout the course of the experiments the stools remained colored and of normal consistency. Three or four weeks after the operations the dogs were all well nourished and showed no disturbances of their gastro-intestinal tracts.

Postmortem examinations in both series of animals showed an obliterated common bile duct with a patent communication between the gall bladder and the stomach in each instance. The gastric mucosa showed no pathological changes.

SUMMARY AND CONCLUSIONS.

It has been shown that small amounts of the digestive secretions normally present in the duodenum regurgitate into the stomach following operative anastomosis between the stomach and the upper intestinal tract. As the duodenal contents are alkaline in reaction this reflux is thought by many to result in a decrease in the acidity of the gastric contents.

The purpose of the investigations reported here has been to determine the effect of bile on the acid chyme of the stomach. By means of a cholecystgastrostomy with ligation and division of the common bile duct in six dogs the bile was diverted from the duodenum into the stomach. The secretion of gastric juice and the acidity of the gastric contents throughout the period of digestion were followed before and after the anastomoses. For this purpose three of the dogs were

provided with Pawlow stomach pouches. In the three remaining animals the test meals were recovered by using apomorphin injections.

The results from the two series of experiments agree. They indicate, at least with a diet of meat and water, that bile when it is present in the stomach throughout the course of digestion has no appreciable effect on the acidity of the gastric contents.

While the anastomosis of the gall bladder to the stomach occasioned some temporary anorexia and vomiting in two of the dogs, the four remaining animals bore the operation well. Three or four weeks subsequent to the operation the digestion and the nutrition appeared normal in each instance.

Postmortem examinations revealed no pathological changes in the gastric mucosa.

BIBLIOGRAPHY.

Boldyreff, W., Der Übertritt des natürlichen Gemisches aus Pankreassaft, Darmsaft und Galle in dem Magen, *Arch. f. d. ges. Physiol.*, 1908, cxxi, 13.

Cannac, L. B., De la cholécystogastrostomie, Thèse de Bordeaux, 1897.

Carle, A., and Fantino, G., Beitrag zur Pathologie und Therapie des Magens, *Arch. f. klin. Chir.*, 1898, lvi, 1.

Collinson, R., Some Present-Day Problems in the Surgery of Gastric and Duodenal Ulcers, *Jour. Am. Med. Assn.*, 1914, lxiii, 1184.

Dastre, A., Recherches sur la bile, *Arch. de physiol. norm. et path.*, 1890, series 5, ii, 315.

Eichmeyer, W., Beiträge zur Chirurgie des Choledochus und Hepaticus einschl. der Anastomosen zwischen Gallensystem und Intestinis, *Arch. f. klin. Chir.*, 1910, xciii, 857.

Finney, J. M. T., and Friedenwald, J., Thirteen Years' Experience with Pyloroplasty, *Surg., Gynec. and Obst.*, 1914, xviii, 273.

Foster, N. B., and Lambert, A. V. S., Some Factors in the Physiology and Pathology of Gastric Secretion, *Jour. Exper. Med.*, 1908, x, 820.

Glaessner, K., Eine neue Therapie der Hyperazidität des Magens, insbesondere bei ulzerösen Prozessen, *Wien. klin. Wchnschr.*, 1913, xxvi, 1557.

Hamburger, W. W., and Friedman, J. C., Contributions to the Experimental Pathology of the Stomach. I. Experimental Pyloric Stenosis, *Arch. Int. Med.*, 1914, xiv, 722.

Hartmann, H., and Lecene, P., Ulcer of the Stomach and of the Duodenum, *Ann. Surg.*, 1914, lx, 227.

Howell, W. H., A Text-Book of Physiology, 5th edition, Philadelphia and London, 1913, 803.

Jaboulay, La cholécystogastrostomie pour les tumeurs de la tête du pancréas, *Lyon méd.*, 1898, lxxxi , 365.

Jacobson, J. H., Anastomosis of the Gall-Bladder to the Stomach: "Cholecystogastrostomy," *Tr. Am. Assn. Obst. and Gynec.*, 1914, xxvii, 172.

Kaiser, O., Über die Erfolge der Gastroenterostomosis, *Deutsch. Ztschr. f. Chir.*, 1901, lxi, 294.

Kausch, W., Ueber funktionelle Ergebnisse nach Operationen am Magen bei gutartigen Erkrankungen, *Mitt. a. d. Grenzgeb. d. Med. u. Chir.*, 1899, iv, 347.

Kehr, H., Die Praxis der Gallenwege-Chirurgie in Wort und Bild, Munich, 1913, ii, 67.

Lönnqvist, B., Beiträge zur Kenntniss der Magensaftabsonderung, *Skand. Arch. f. Physiol.*, 1906, xviii, 194.

Masse, M., Cholecystogastrostomie, *Rev. de chir.*, 1898, xviii, 1122.

Mayo, W. J., Chronic Ulcers of the Stomach and Duodenum, *Ann. Surg.*, 1914, lx, 220.

Mocquot, P., Anastomoses de la vésicule biliaire avec l'estomac et avec le duodenum, *Compt. rend. Soc. de biol.*, 1911, lxxi, 118.

Noetzel, A., Communication to Eliot, *Ann. Surg.*, 1912, lv, 709.

Oddi, R., Physiology of the Digestive Glands, *Centralbl. f. Physiol.*, 1887–88, i, 312.

Palfrey, F. W., The Administration of Ox Bile in the Treatment of Hyperacidity and of Gastric and Duodenal Ulcer, *Am. Jour. Med. Sc.*, 1913, cxlv, 796.

Pawlow, I. P., The Work of the Digestive Glands, London, 1910, 108.

Pawlow, J. P., Die physiologische Chirurgie des Verdauungskanals, *Ergebn. d. Physiol.*, 1902, i, pt. 1, 246.

Pfaff, F., and Balch, A. W., An Experimental Investigation of Some of the Conditions Influencing the Secretion and Composition of Human Bile, *Jour. Exper. Med.*, 1897, ii, 49.

Rywosch, D., cited by Smith, G. M., An Experimental Study of the Relation of Bile to Ulceration of the Mucous Membrane of the Stomach, *Jour. Med. Research*, 1914, N. S., xxv, 147.

Smith, G. M., An Experimental Study of the Relation of Bile to Ulceration of the Mucous Membrane of the Stomach, *Jour. Med. Research*, 1914, N. S., xxv, 147.

Wiedemann, H., Experimentelle Untersuchungen zur Lehre der Verdauung und Resorption verschiedener Nahrungsprodukte bei anormalem Gallenzufluss in den Verdauungsapparat, *Beitr. z. klin. Chir.*, 1914, lxxxix, 594.

A PATHOLOGICAL STUDY OF SYPHILITIC AORTITIS AND ITS SEROLOGY.

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Plates 1 to 7.

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The Wassermann reaction as a diagnostic factor in syphilis holds a unique position in clinical medicine. There are those who believe it an infallible method for proving the presence of syphilis, but there are as many who are skeptical about accepting a serological diagnosis. Neither the serologist nor the clinician can hope definitely to ascertain the value of the complement fixation test in lues. As in all other problems in medicine, it is the work of the pathologist to demonstrate the probable focus of infection in an individual with a positive Wassermann reaction, in whom there is no clinical evidence of syphilis. With this in view it occurred to us to check the serological diagnosis on the autopsy table; that is, to find some definite pathological explanation for this type of reaction in so called latent syphilis. Since syphilis primarily attacks the blood vessels—in fact, it is regarded as a disease the manifold pathological changes of which have their origin in diseased blood vessels—we turned our attention first to the aorta.

Though the pathology of specific aortitis is fairly well understood and its various phases have been accurately described, and though there is a classical method of performing the Wassermann reaction, the problem presents many difficulties. Luetic aortitis, grossly and microscopically, presents many characteristic features, so our task is chiefly to point out what are the primary and early changes of a syphilitic process in the aorta. Since numerous modifications of the original Wassermann method are employed in doing the reaction, it is essential to study and to determine a certain routine serological

procedure, which, with the greatest degree of accuracy, will diagnose the presence or absence of specific changes in the aorta.

We obtained aortas from every case autopsied in the past four years in the Strecker Memorial Laboratory on which a serological examination of the blood had been made before death. Unfortunately, this includes only a small number of cases sectioned. With the aid of serology we have been able to point out definitely that the diagnostic lesion of specific aortitis is a histological one, and in the routine examination of a series of aortas a surprising number reveal this lesion in varying degree, without giving any gross evidence of syphilitic infection.

Gross Appearance.

In most instances in those who have died of aortitis the gross appearance of the aorta presents a typical picture. The lesion is confined to a definite portion of the aorta, usually the ascending and transverse parts, with a sharp line of demarcation between the lesion and the remainder of the aorta, which appears fairly normal (Fig. 3). The involved portion presents either a diffuse dilation or a saccular aneurysmal pouch, and the normal glistening appearance of the intima is lost, being replaced by confluent, pearly white, elevated scar areas, scattered through which are often seen yellowish patches of fatty degeneration (Figs. 1 and 2). To the touch it is hard, thickened, and fibrous. Though this gross appearance is characteristic, it is by no means diagnostic. In our series of seventeen cases eleven died of aortitis, and a little over one-half of these presented this typical picture; the others showed either a diffuse process, the dominating feature of which was an athero-degenerating sclerosis, superimposed upon a syphilitic process, or only slight changes in the intima were appreciable.

Microscopical Changes.

Chiari, in 1903, gave an excellent description of the microscopical changes. He calls attention primarily to the striking appearance of the media, in which one notes small areas of granulation and fibrous tissue, often with central necrotic zones and newly formed vascular elements, surrounded by infiltrating round cells. He also pointed out that in the adventitia the vasa vasorum were

distinctly thickened, and that a perivascular, round cell infiltration was associated with these changes in the adventitia. He regarded the changes in the intima as secondary, and presenting nothing characteristic of a luetic process, but showing various degrees of a simple atherosclerosis. Chiari's description, however, is only typical of an advanced case of luetic aortitis. It is the histological picture seen in the aortas taken from those who have died of the disease. We feel confident, from a study of the material at hand, that an earlier process of luetic aortitis presents quite a different picture. A productive inflammation in the aorta to our mind is pathognomonic of syphilis, and histologically it can easily be differentiated from the simple degenerating process in atherosclerosis. The earlier changes we have seen in aortas from individuals dying of tabes or syphilitic meningomyelitis, and, in several instances, from a non-syphilitic disease. The adventitia, undoubtedly, is the site of predilection, and here one sees the earliest evidence of a productive inflammation. Grouped around the slightly thickened vasa vasorum there is a distinct round cell infiltration. Associated with this perivascular inflammatory reaction the adventitia appears thickened; the fibers are coarser and heavier, and the thickening is directly proportionate to the degree of infiltration. It is important to note in this early stage that the inflammatory process is usually confined to the adventitia, and that the medial coat presents no evidence of a fibrous or lymphoid infiltration. In many instances, however, with a moderate perivascular infiltration in the adventitia the media and intima may be the seat of a marked degenerative process.

From a thorough study of a number of syphilitic aortas it is evident that a perivascular round cell infiltration is a constant feature of the diseased process. The typical cells closely resemble lymphocytes, small round cells staining intensely with a nuclear stain. With these one sees other types,—endothelial and plasma cells—but these are by no means invariably present (Fig. 7). Many have found giant cells in the infiltrations in the media, and a few have called attention to their presence in the adventitia, but in our series we were unable to observe any in our microscopical preparations. Gruber seldom found them and agrees with Thorel, that one may be unable to see giant cells, even in the examination of a large number of cases.

Perivascular Cellular Infiltration as a Diagnostic Feature of Luetic Aortitis.

That this histological lesion in an aorta, namely, perivascular cellular infiltration, has syphilis as its etiological factor, and further, that it is diagnostic of luetic aortitis, we base our opinion upon the following facts.

Eighteen of the forty-two aortas examined presented this picture, and seventeen of these gave a positive Wassermann during life. In other words, 94 per cent of the aortas showing a round cell infiltration gave a positive Wassermann reaction. On the other hand, twenty-four aortas presented no evidence of a perivascular infiltration, and twenty-two of these gave negative, while two gave positive reactions; that is, 91 per cent of these aortas gave negative complement fixations. Gruber working with postmortem serum in a series of 106 cases of syphilitic aortitis obtained identical results, 94.3 per cent positive fixations, and he states that any evidence of a productive inflammation in the aorta with a positive Wassermann gives us at least 0.9 per cent assurance that this type of inflammation is a result of syphilitic infection, even though the dominating feature is one of deforming atherosclerosis.

Such lesions as ruptured aneurysm, and aortitis with pure aortic lesions, undoubtedly syphilitic in origin, histologically present this in a marked degree. In other general metabolic and infectious processes, such as arteriosclerosis, interstitial nephritis, chronic ulcerative tuberculosis, carcinoma, pneumonia, vegetative endocarditis, and pernicious anemia, the aortas showed no such histological picture.

Most authorities, however, do not agree that a perivascular, round cell infiltration in the aorta is characteristic and pathognomonic of luetic infection. Stadler maintains that moderate infiltration around the vasa vasorum may be due to alcohol and to certain infections, and Mönckeberg describes it in a case of recurrent endocarditis with rheumatic joint lesions. Gruber admits that syphilis can cause only a productive inflammation with the typical gross changes in an aorta, but that an inflammatory infiltration may occasionally, though seldom, be caused by other processes, and while histologically he is unable to determine the etiological factor, the serological reaction gives him the diagnosis. Gruber agrees with Faber, that though the dominant feature of atherosclerosis is a degenerative process with calcification, ulceration, and deformity, undoubtedly a moderate inflammatory reaction is occasionally met with in this type of sclerosis. We will admit that fibrous connective tissue and thickened vasa vasorum surrounded with fibroblastic cells are occasionally seen in atherosclerosis, and that this is a chronic productive inflammation,

but that the typical round cell perivascular infiltration, already described, is a luetic process.

That infectious diseases, such as colon bacteremias or streptococcic endocarditis, are a possible cause of lymphoid infiltration in an aorta does not coincide with our experience. In two cases of vegetative endocarditis, and in one of pyonephrosis with colon infection, the aortas showed no inflammatory changes, and the serology was negative. In another, however, with focal necroses (a streptococcic infection) the aorta showed moderate perivascular infiltration, but the serology was positive, and the infection was secondary to an ulcerating gumma of the larynx. Gruber has noted two interesting cases of inflammatory infiltrations around the vasa vasorum in the adventitia of the aorta, secondary to an inflammatory process in the para-aortic tissue. In one case tubercular lymph glands in the mediastinum had ulcerated, producing a marked inflammatory reaction. In the other, perforation of a stenosis of the esophagus through surgical interference, from which the patient died several months later from sepsis, produced a typical productive inflammation in the aorta, which could not be differentiated from a luetic process. Both these cases gave negative Wassermann reactions, and Gruber concludes that they were non-specific. That a lymphoid infiltration and productive inflammation may result as an extension process is undoubtedly true, but that does not alter our opinion that this type of reaction produced in an aorta *per se* is pathognomonic of syphilis.

Treponema pallidum in Luetic Aortitis.

Though serology gives us definite proof that syphilis produces a typical aortitis, as further evidence in support of our view, we have attempted to find *Treponema pallidum* in our preparation. Wright and Richardson, with others, claim to have demonstrated the spirochæta in the aortas of acquired syphilis, but we have been unsuccessful in doing so. We have seen many artefacts in our preparations, which, by less skeptical men, could easily be interpreted as spirochætæ. Both Gruber and Fukushi have also failed to find the spirillum in their large series of cases.

Classification of Cases.

So, basing our diagnosis upon a round cell perivascular infiltration we have in our series eighteen cases showing evidence of syphilitic aortitis. We have divided this series into three classes: first, those showing this in a marked degree; second, those cases in which the infiltration is moderately present; and third, those in which there is only slight evidence of a cellular infiltration (Figs. 4, 5, and 6). In Class 1 there are eleven cases. Over one-half of the aortas presented the gross picture of specific aortitis; the rest showed a diffuse atheromatous process, with areas of ulceration. All these cases died of aortitis; three with ruptured aneurysm, the others with symptoms of cardiac decompensation. Five of the cases had marked aortic valve involvement, with normal mitral valves, or slightly thickened mitral cusps. One case had both valves involved. There was positive serology in all cases.

In Class 2, a series of five cases, none presented the gross typical picture. Three of the aortas were markedly atheromatosed, with areas of calcification and ulceration. The two others were fairly normal, with the exception of longitudinal striations and thickened, raised, whitish areas around the intercostal orifices. Histologically, however, the perivascular infiltration was distinctive and definite. In only one case was the aortic valve diseased. None of these cases died of their aortitis. One died with symptoms of cardiac decompensation, probably from localized fibrous infiltration of the wall of the left ventricle, involving the area of the bundle of His, with aneurysmal dilatation at this point. The others died of the following conditions: syphilitic meningomyelitis, carcinoma of the right bronchus, chronic ulcerative tuberculosis, and terminal bronchopneumonia with tabes. The serology was positive in all but one case.

In the third class, consisting of only two cases, characterized by a very moderate or slight infiltration around the vasa vasorum the aortas appeared practically normal, with the exception of patches of smooth, elevated areas around the intercostal orifices. One case died of carcinoma of the bronchus, the other of an ulcerating gumma of the larynx, with focal necroses of kidney and liver. Both cases had a positive serology.

Complications.

The most common complication of syphilitic aortitis is an insufficient aortic valve. In a study of forty-two cases with various types of aortas the aortic valve in fifteen showed evidence of disease. In seven of these fifteen valvular lesions the mitral valve was normal, and the aortas of these seven cases, in which only the aortic valves were involved, gave marked evidence of luetic aortitis, and the serology was positive, with the exception of one. In this case the aortic valve was practically destroyed with a vegetative ulceration; the serum was negative and the aorta normal. Of the remaining eight cases both valves were diseased; six consisting of fibrous changes, and two having acute vegetations. In only one aorta with thickened fibrous changes in both valves was there evidence of luetic aortitis, and in this case also the serum was positive. The seven other cases showed no productive inflammation in the aorta, and the complement fixation tests were negative. From this it seems evident that pure aortic insufficiency, with the exception of infectious endocarditis, is undoubtedly of syphilitic origin. With both valves involved the probability of a syphilitic infection of the aorta is small; an atheromatous or an infectious process should suggest the probable origin.

Luetic aortic insufficiency is, in most cases, probably a late process. In our seven cases of syphilitic involvement of the aortic valve, six were a complication of those specimens which showed marked histological changes in the aorta (Class 1) and all of these died of the vascular lesion. In other words, six out of ten specimens of late aortitis, or 60 per cent of the cases of advanced aortitis, had the aortic valve involved. On the other hand, of the seven cases of early luetic changes the aortic valve was part of the process only once, giving in our whole series the complication in about 40 per cent of the cases. This practically agrees with Stadler's findings, in which he states that while only one-third of his luetic aortas were complicated with a diseased valve, two-thirds of his cases dying of aortitis had insufficient aortic valves.

Ruptured aneurysm is a serious, though not so frequent a complication. Of course it is met with only in advanced cases. In our series of eleven dying with aortitis, three succumbed to a rupture of

an aneurysm. In two the rupture was in the thoracic cavity. In one the aneurysm was abdominal and had eroded the vertebra almost to the cord.

Cerebral luetic endarteritis is a complication of syphilitic aortitis as often as is aortitis. We can only consider cerebral endarteritis a complication of the aortitis when there are symptoms of a brain lesion, and evidences of brain softening in an individual dying of aortitis. Conversely, in individuals dying of their brain lesions the endarteritis is complicated with an aortitis, when there are either clinical or pathological evidences of aortic disease present. In our eleven cases of fatal aortitis two individuals had evidences of paralysis before death, and in these cases areas of brain softening were demonstrated. In seven cases of early aortitis only one died of cerebral softening.

Tabes complicates specific aortitis less often than it is complicated by the vascular lesion. In over ten fatal cases none gave during life any clinical evidence of tabes. It is of interest to note here, however, that the histological examination of the cord in one case revealed an early sclerosis of the posterior root fibers, the posterior tract of the cord not being affected. In two fatal cases of tabes diffuse atheroma with calcification and ulceration were present in both aortas. In one, however, there was a moderate perivascular infiltration, and the serum in this case was positive; the other gave no evidence of syphilis in the aorta or in the serum. Stadler found clinically in 248 cases of aortic disease that 6.2 per cent of these had tabes, and he further states that in his autopsy findings almost all cases of tabes showed syphilitic aortitis.

Aortitis is a frequent complication of general paresis. Gruber and Straub at autopsy found that 71 and 82 per cent, respectively, of their cases of general paresis showed evidence of luetic aortitis.

Stadler found narrowing of the lumen of the mouth and sclerosis of the coronaries in one-third of his autopsies on aortitis. This is undoubtedly true, but it is difficult to differentiate the dominating factor producing this change, whether an atherosclerosis or a productive inflammation. It is questionable, as the older writers have suggested, whether the earliest changes in syphilis of the aorta are seen around the orifice of its branches, producing a narrowed lumen.

Only occasionally have we noticed in advanced aortitis anything approaching a definite stenosis of these orifices.

In over two-thirds of our cases there was no cardiac enlargement (Figs. 1 and 2). This agrees with Grau's findings, but Gruber in a majority of his specimens reports a distinct hypertrophy of the heart. Some have stated that cardiac hypertrophy is associated with the aortic insufficiency, but in some of our most marked cases of aortic disease we have found surprisingly small hearts. It seems, however, that cardiac hypertrophy is not associated with luetic aortitis or any of its complications, but is a result probably of some type of nephritis.

Serology.

To discuss technically the serology of syphilitic aortitis is not the scope of this paper, but rather to point out that in our hands, at least, a definite serological procedure is preferable to either the original method, or several popular modifications, in diagnosing syphilis of the aorta, and that a high percentage of positive reactions in individuals who give no clinical evidence of syphilis of the vascular system can be explained by luetic aortitis.

Wassermann Reaction as a Diagnostic Factor in Luetic Aortitis.

As stated, forty-two aortas have been examined and the sera of these cases tested serologically before death. Nineteen of these gave positive Wassermann tests; twenty-three were negative. Of the nineteen positive cases, seventeen aortas gave microscopical evidence of luetic aortitis; namely, 90 per cent of our positive reactions (those sera fixing complement according to the method above described) have, at least, a definite pathological explanation based on changes in the aorta which are pathognomonic of syphilis. It is interesting to note that fifteen of the nineteen cases died as a result of a syphilitic process (about 80 per cent), while eleven of the nineteen died from their luetic aortitis (about 60 per cent). The four cases dying from syphilis other than aortitis were one case of syphilitic cerebral endarteritis, one of tabes, one of meningomyelitis, and one ulcerating gumma of the larynx. Of the four cases with positive Wassermanns dying of non-syphilitic conditions but showing perivascular infiltra-

tion in the aorta, two died of carcinoma of the bronchus, one of chronic ulcerative tuberculosis, and one of interstitial nephritis.

Though the cases studied are too few to make any definite deduction, they are suggestive. It is highly probable that about 90 per cent of individuals dying with a positive Wassermann, if the infection is not of recent origin, have, at least from the histological point of view, luetic aortitis, that about 60 per cent of them die from their aortitis, giving clinical evidence of cardiac decompensation or rupture of an aneurysm, and that about 80 per cent of these individuals die of syphilis. Since only one case of perivascular cellular infiltration gave a negative Wassermann test in our series, it is fair to assume that 94 per cent of individuals suffering from luetic aortitis would give a positive reaction in their serum.

That our results correspond with the data in the literature is shown by the recent works of Stadler and Gruber. Gruber obtained identical serological reactions in his series of luetic aortitis; namely, 94.3 per cent positive Wassermanns on postmortem serum. Basing his results on the pathological findings, Stadler states that 82 per cent of 256 cases of syphilis had aortitis, and so considering a positive fixation as diagnostic of syphilis we find, as stated, that 90 per cent of syphilitics have evidence of aortitis. Our higher figure could easily be accounted for by considering (as we did) every aorta with perivascular infiltration as specific. Though only 46 per cent of Stadler's cases died of their vascular lesion, in our series we found that the aortic lesion accounted for deaths in 60 per cent.

The serology of aortic insufficiency is confusing. Serologists obtain positive reactions ranging anywhere from 50 to 80 per cent of the cases. The reason for this large discrepancy is due to the fact that the clinicians have failed to differentiate between the various types of this lesion. If one remembers that only pure aortic insufficiency, with the exception of an infectious endocarditis, is always syphilitic, and that the serology is positive in probably 100 per cent of the cases, and that the aortic lesion associated with a mitral incompetent valve is usually an atherosclerotic process, and only occasionally specific, one can readily understand the disagreement in the serological results.

Wassermann Reaction in Treated Cases.

The effect of treatment on the serology of luetic aortitis should receive passing notice. All the cases with varying amounts of treatment, including salvarsan and mercury, with the exception of four, remained positive throughout. The serology was modified in four cases, as follows: No. 292 was admitted with a positive Wassermann reaction; salvarsan was given, followed by several doses of mercury, and following this the Wassermann was negative. No. 678 was admitted with a weakly positive reaction; that is, 50 per cent hemolysis on the guinea pig antigen. Before admission the patient had received several doses of mercury. Without treatment at the end of a month the serology was strongly positive. Five salvarsan injections were given, and a month later the Wassermann test was still positive. A year later, however, just before death, the serology was reported doubtful; since, though there was complete inhibition of all antigens at the first reading, at the end of twelve hours there was complete hemolysis, even with cholesterinized antigen. No. 694 was admitted with serology noted as weakly positive, 50 per cent hemolysis on guinea pig heart antigen. Mercury had been given before admission. Eight months later (three months after the salvarsan and mercury treatment) the serum was reported positive. No. 691, which was admitted with a positive reaction after one salvarsan and several mercury injections, was reported as negative.

A careful review of these cases notes the importance in serologically diagnosing luetic aortitis of repeating the reaction on sera, especially if any form of antispecific treatment has been administered. Though in 75 per cent of the cases treatment did not alter the serological reaction in the blood, in 25 per cent it either weakened the reaction or produced a negative fixation.

Technique and Antigens.

In this series a positive reaction means complete inhibition of hemolysis when the controls have hemolyzed, and at the end of twelve hours. The antigen employed is an alcoholic extract of guinea pig heart. 0.1 cc. of complement and at least two units of amboceptor are used in the hemolytic system. Many of the sera were

done with other antigens; namely, alcoholic extract of syphilitic liver, acetone partition of calf's heart, cholesterinized alcoholic extract of human heart and guinea pig heart. But the findings with these various antigens were not as constant and as consistent as with the first named extract. We do not mean to infer that these extracts are not to be used in the Wassermann reaction, but rather that in the serological diagnosis of syphilitic aortitis, the alcoholic extract of guinea pig heart should receive first consideration.

The antigens made from syphilitic fetal livers were unreliable with us, while acetone partition antigens made according to Noguchi's method, though giving fairly good results, were influenced earlier by treatment and the completed extracts seldom were of uniform strength. These antigens have been discarded by us in reporting the routine Wassermann reaction in the laboratory. It is important to note, however, that Gruber working with fetal syphilitic liver antigens obtained results identical with ours. Our experience with the liver antigen (that it is so unreliable) is not surprising, since, though we attempted to obtain congenital luetic organs, in many instances we were not assured by spirochæta demonstration that our material was from a syphilitic infant. The highly sensitized cholesterin antigens we still employ with the assurance that complete complement fixation with this antigen probably does not always indicate the presence of syphilis. On the other hand, however, many other types of syphilis, such as tabes, give only positive reactions with this antigen.

Though 90 per cent of our positive reactions with guinea pig heart antigen showed evidence of syphilis in the aorta, only 77 per cent of the positive results with cholesterin could be explained by similar luetic changes.

Finally, we hope that these facts will be of assistance to the clinician in diagnosing syphilis of the aorta, and further, that they will emphasize the importance of a positive Wassermann reaction, and make it incumbent upon all physicians to institute treatment as a prophylactic measure in such cases.

Syphilis is an endemic infectious disease which ranks with tuberculosis and carcinoma as a menace to the community, and which, when untreated, probably kills 80 per cent of its victims and produces an aortitis in about 80 to 90 per cent, of whom 50 or 60 per cent die of the vascular lesion.

SUMMARY.

1. Syphilitic aortitis is a productive inflammatory process, the earliest and most constant feature of which is a perivascular round cell infiltration in the adventitia.

2. The typical gross picture of luetic aortitis is often obscured by a superimposed, diffuse atherosclerosis. In the early cases the aorta appears fairly normal, presenting only the characteristic histological changes.

3. A pure aortic insufficient valve, with the exception of an infectious endocarditis, is always luetic.

4. Cardiac hypertrophy is not a complication of luetic aortitis. When present it is usually associated with a nephritis.

5. The demonstration of *Spirochæta pallida*, even in advanced specimens of syphilitic aortas, is doubtful.

6. An antigen prepared from alcoholic extract of guinea pig heart with the original Wassermann technique should be preferred in diagnosing luetic aortitis.

7. Positive complement fixations in patients suffering with syphilis for a period of about fifteen years or longer suggest the probability, at least, of histological luetic changes in the aorta in 80 to 90 per cent of the cases. 60 per cent of these die from aortitis.

8. About 94 per cent of patients suffering with aortitis give positive Wassermann reactions.

BIBLIOGRAPHY.

Chiari, H., Ueber die syphilitischen Aortenerkrankungen, *Verhandl. d. deutsch. path. Gesellsch.*, 1904, vi, 137.

Faber, A., Arteriosklerose. Ihre pathologische Anatomie, ihre Pathogenese und Ätiologie, 2d edition, Jena, 1912.

Fukushi, M., Die pathologische Anatomie der syphilitischen Aortitis mit besonderer Berücksichtigung des Vorkommens von Plasmazellen, Berlin, 1914.

Grau, H., Über die luetische Aortenerkrankung, *Ztschr. f. klin. Med.*, 1911, lxxii, 292.

Gruber, G. B., Über die Doehle-Heller'sche Aortitis, Jena, 1914.

Mönckeberg, J. G., Ueber die Beziehungen zwischen Syphilis und schwieliger Aortensklerose vom pathologisch-anatomischen Standpunkt, *Med. Klin.*, 1915, i, 1027.

Stadler, E., Die Klinik der syphilitischen Aortenerkrankung, Jena, 1912.

Thorel, Ch., Pathologie der Kreislauforgane, *Ergebn. d. allg. Path. u. path. Anat.*, 1911, xiv, pt. 2, 681.

Wright, J. H., and Richardson, O., Treponemata (Spirochætæ) in Syphilitic Aortitis: Five Cases, One with Aneurism, *Boston Med. and Surg. Jour.*, 1909, clx, 539.

EXPLANATION OF PLATES.

Plate 1.

Fig. 1. Note the small heart with concentric hypertrophy. The aortic valves are moderately thickened. The walls of the aorta are markedly thickened. There is a diffuse dilatation of the ascending and transverse portions of the aorta, and a marked fibrous appearance of its inner wall.

Plate 2.

Fig. 2. The same as Fig. 1, but showing aneurysmal formation and involvement of the aortic valves. In both Fig. 1 and Fig. 2 the mitral valves were normal.

Plate 3.

Fig. 3. Portion of a syphilitic aorta showing a definite line of demarcation between involved and normal parts at the junction of the lower third.

Plate 4.

Fig. 4. Microscopical picture of a section of the aorta of the case shown in Fig. 1. Note the perivascular infiltration of the vasa vasorum, the thickened adventitia, the newly formed vascular elements in the media, with its round cell infiltration, also the thickened intima,—a secondary process.

Plate 5.

Fig. 5. The same as Fig. 4, but not so advanced.

Plate 6.

Fig. 6. An early process with a beginning atherosclerosis.

Plate 7.

Fig. 7. A high power picture showing the type of cells. Note the great number of lymphocytic cells and several larger plasma cells.

FIG. 1.

(Larkin and Levy: Syphilitic Aortitis.)

FIG. 2.

(Larkin and Levy: Syphilitic Aortitis.)

FIG. 3

(Larkin and Levy: Syphilitic Aortitis.)

FIG. 4.

(Larkin and Levy: Syphilitic Aortitis.)

FIG. 5.

(Larkin and Levy: Syphilitic Aortitis.)

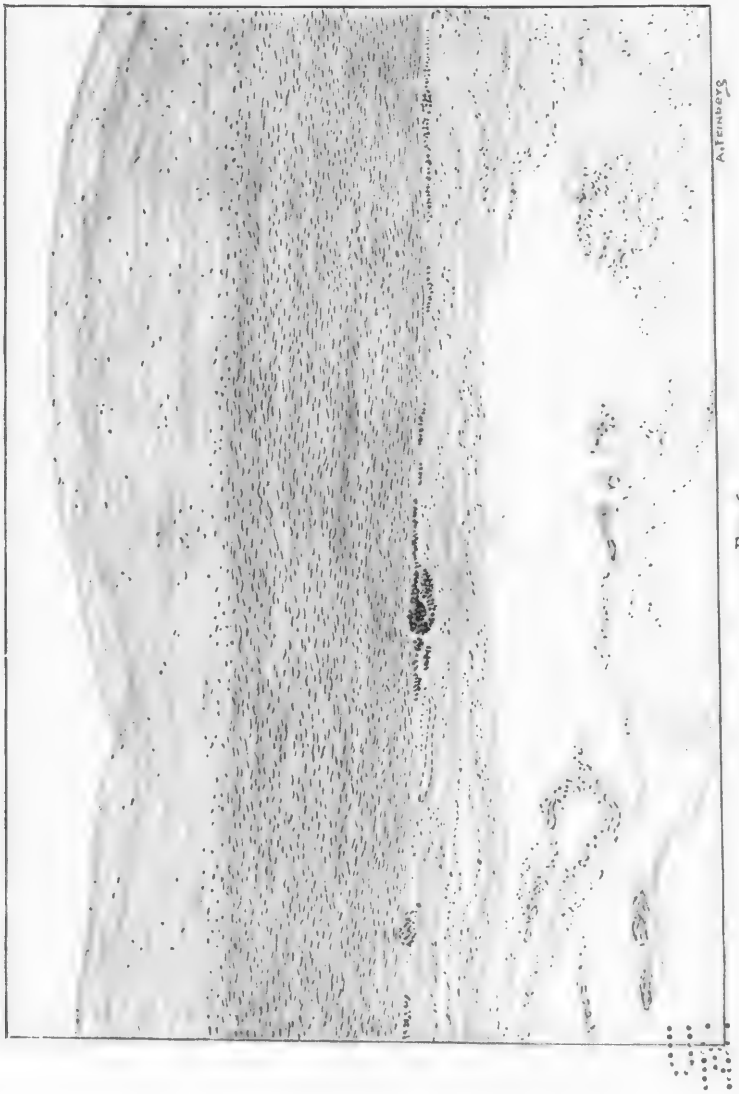

FIG. 6.

(Larkin and Levy: Syphilitic Aortitis.)

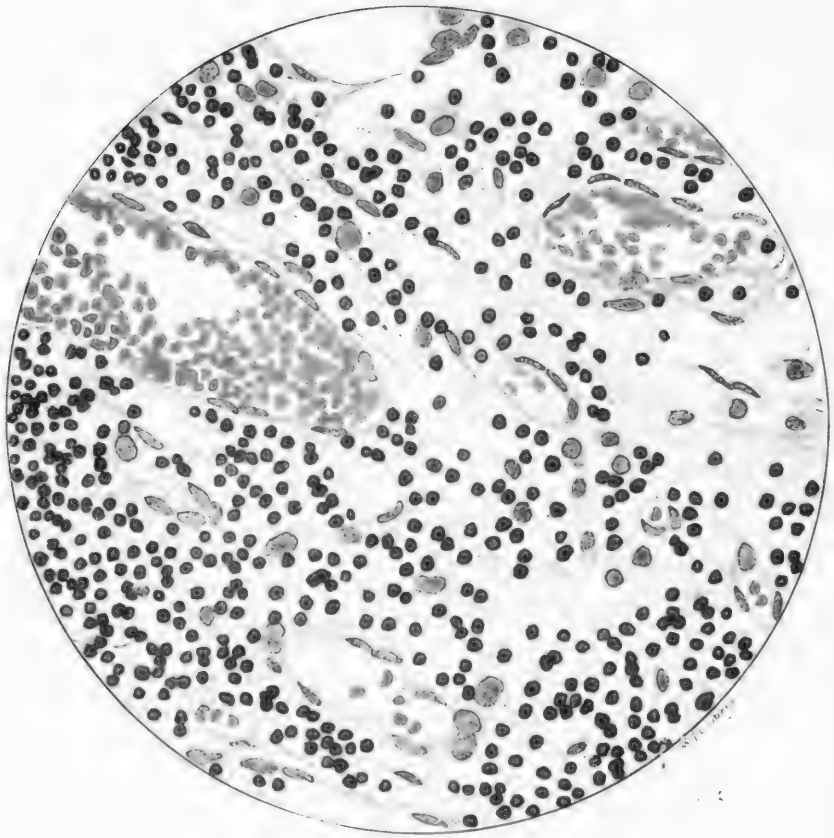

FIG. 7.

(Larkin and Levy: Syphilitic Aortitis.)

THE ETIOLOGY OF RAT-BITE FEVER.

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Plates 8 to 14.

(Received for publication, September 23, 1915.)

Rat-bite fever, or Sodoku, has long been recognized in Japan as a definite febrile disease, following the bite of a rat.

Miyake (1) in 1899 was the first to describe the disease in detail as a definite clinical entity and reported eleven cases of his own with references to others in the Japanese literature. For many years reports of sporadic cases have appeared in America, the first being recorded as early as 1840 by Wilcox (2). In 1909 Horder (3) reported three cases from England, and since that time instances of the disease have been recorded with increasing frequency in the British literature. The incidence of rat-bite fever in continental Europe seems to have been less frequent. Cases have been recorded in France by Millot-Carpentier (4), De Micas (5), and Curtillet and Lombart (6); in Spain by Peña y Maya (7); in Italy by Frugoni (8); and in Germany by Schottmüller (9).

The clinical characteristics of rat-bite fever have been well presented in papers by Miyake (1), Horder (3), Ogata (10), Atkinson (11), Gouget (12), Proescher (13), and Frugoni (8). A comprehensive description of the disease with report of a new case and summary of 53 cases collected from the literature has recently been published by Crohn (14). To the cases collected by him may be added the following, making a total of 81 cases: in America, Farquhar (15)[1] 6; in the British Isles, Atkinson (11) 6; Nixon (16) 1; Dick and Rutherford (17) 1; in France, Curtillet and Lombart (6) 1; in Spain, Peña y Maya (7) 1; in India, Dalal (18) 1; in Japan, Ogata (19, 20) 9; and in Germany, Schottmüller (9) 1.

None of these cases with the exception of that reported by Schottmüller (9), which will be discussed below, has added anything new to our knowledge of rat-bite fever, so that it does not seem of value to review them in detail.

In view of the recent publication by Crohn (14) the clinical features of rat-bite fever will be given only in brief and the more detailed discussion will be devoted to the etiology and pathology of the disease.

Rat-bite fever is a paroxysmal febrile disease of the relapsing type following the bite of a rat. The wound heals readily, but after a variable incubation period of a few days to a month the wound becomes inflamed and painful. Lymphangitis and adenitis set in and are quickly followed by symptoms of systemic infection

[1] Farquhar's report is too brief to make the diagnosis certain in his cases.

ushered in by a chill and rapid rise in temperature. There is extreme prostration, severe generalized muscular pain, headache, weakness, and loss of appetite. Stupor, delirium, and even coma may supervene. There is muscular tenderness and rigidity and the tendon reflexes are frequently exaggerated. A characteristic exanthem of bluish red, erythematous, sharply marginated macules appears, varying in size from 1 to 10 cm. in diameter and of general distribution. After 5 to 9 days the temperature falls by crisis accompanied by a drenching sweat and all symptoms subside. The disease then assumes the relapsing type with paroxysms occurring at fairly regular intervals, usually about once a week. The course may vary from one to three months or even longer. Gradually the relapses become less frequent and less severe and the disease often terminates with an abortive paroxysm. The more important complications are nephritis, severe anemia, and emaciation. About 10 per cent of the cases terminate fatally, usually during the first febrile period, occasionally later from nephritis or exhaustion.

That rat-bite fever is an infectious disease dependent upon the introduction of a specific organism or virus at the site of the bite seems highly probable. There has been no definite evidence presented to show that the rat inflicting the bite was in any way diseased or other than a normal rat. In view of this negative evidence it does not seem improbable that the etiological agent is simply a saprophyte in the mouth of the rat. Individual susceptibility seems to play a definite part in the contraction of the disease, as instances are recorded in which the second of two people bitten by the same rat has been the only one to develop rat-bite fever (Miyake (1)). Nixon (16) has reported a case similar to rat-bite fever following the bite of a ferret, and Schottmüller (9) a case incident on the bite of a South African squirrel. That the etiological factor in such cases is closely related to that occurring in rat-bite fever is possible.

The relapsing type of the fever has suggested that the disease may be of protozoan or spirochetal origin, but there are no facts to substantiate this supposition.

Most of the cases reported during the last decade have been carefully studied bacteriologically without yielding definite results until the recent case of Schotmüller (9) reported in 1914. Ogata (10, 19) in a series of eight cases has described parasitic organisms in the blood, excised lymph nodes, and smears from the local lesions. He considered the organism to be a sporozoon and has observed various stages in its development,—sporozoites, merozoites, etc. Guinea pigs and rabbits inoculated with material from his cases died in 18 to 21 days. The same organism was observed in the blood and smears from lymph nodes, liver, and spleen of the experimental animals. In a subsequent communication Ogata (20) has reported on his findings in three additional cases. The same organisms were described in the blood and lymph nodes, and in addition mycelial threads were observed. The organism was cultivated at room temperature on gelatin and agar slants from blood obtained from the finger tips of his patients. Animal experimentations yielded the same results as in the former cases. It is to be noted, however, that he has changed the classification of his organism and con-

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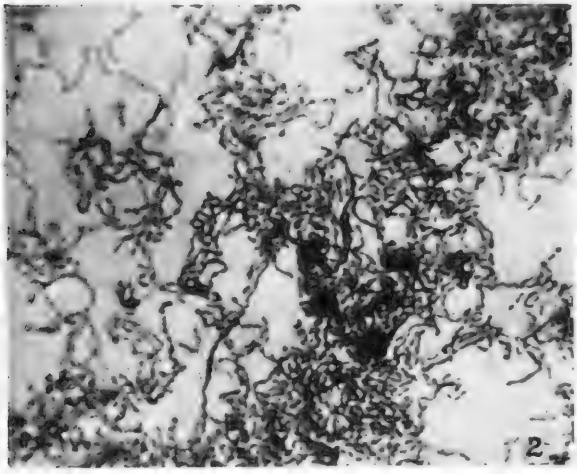

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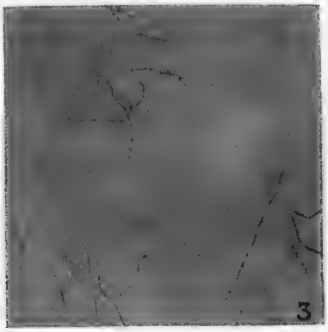
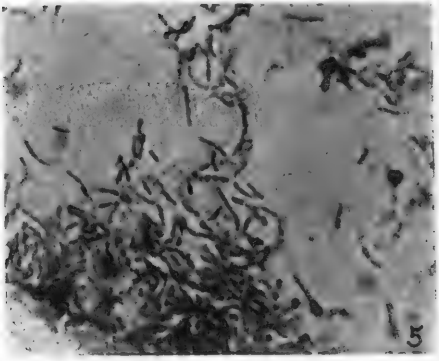
(Blake: Etiology of Rat-Bite Fever.)





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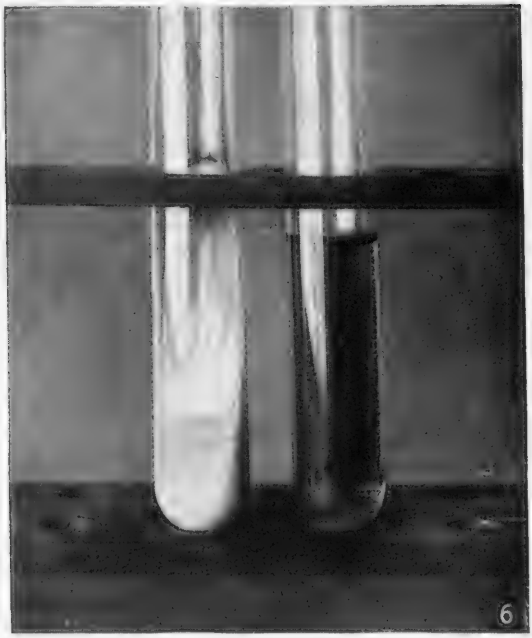
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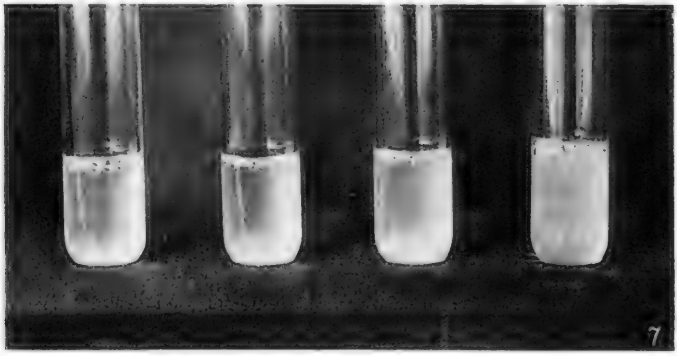
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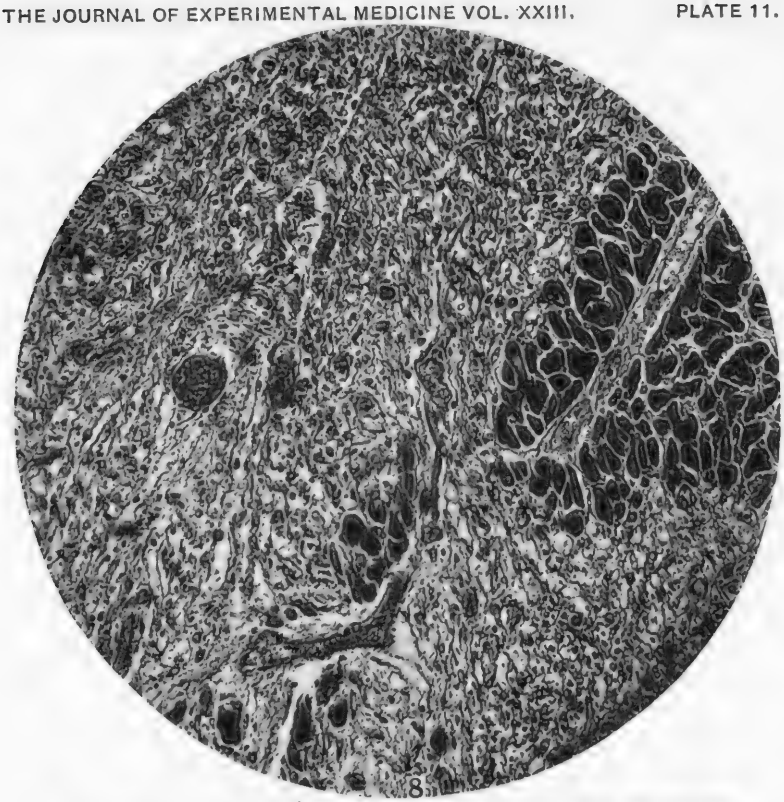

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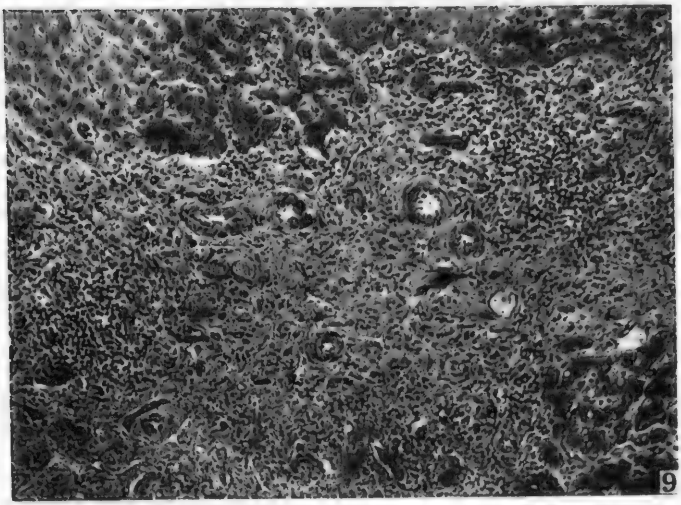

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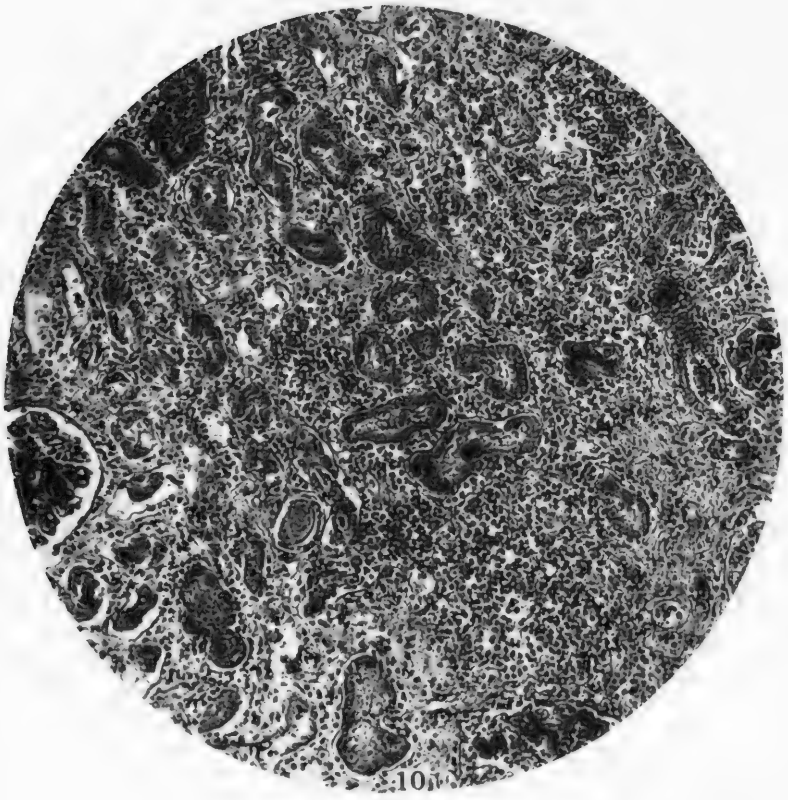
(Blake: Etiology of Rat-Bite Fever.)



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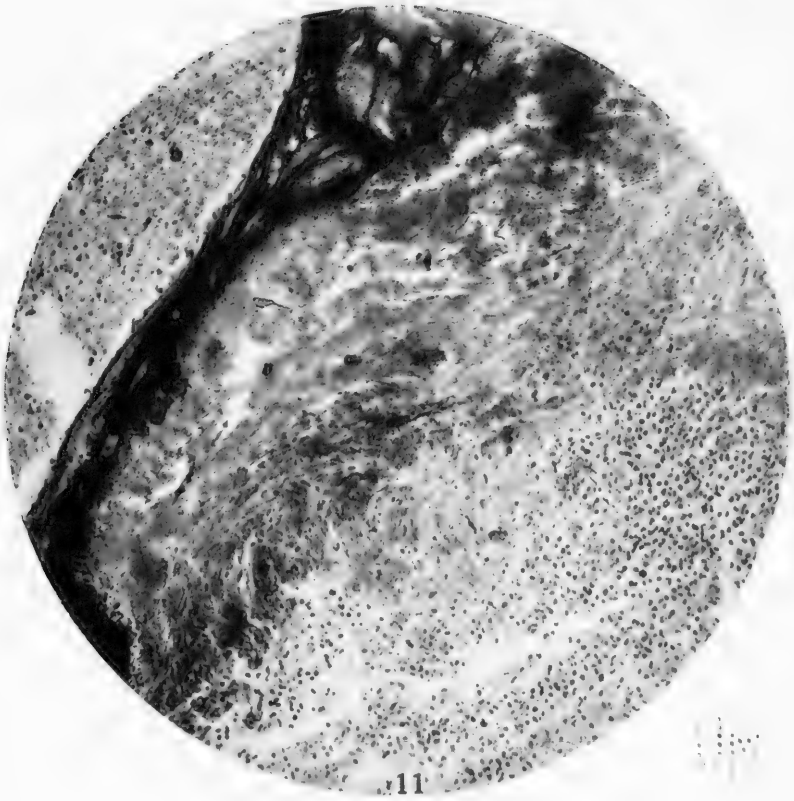

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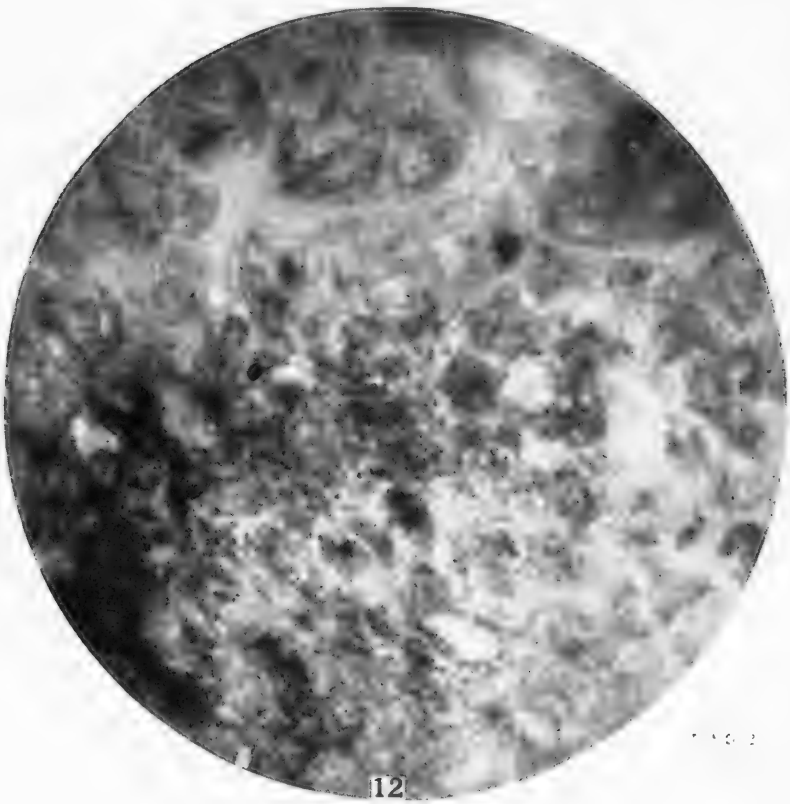


(Blake: Etiology of Rat-Bite Fever.)

siders it to be an aspergillus, basing his conclusions on the cultural characteristics and appearances in smears prepared from experimental animals. Ogata's work has not been confirmed by other observers.

Middleton (21) in his case noted rod-like organisms in fresh blood smears, and one colony of a non-capsulated diplococcus appeared in an anaerobic gelatin blood culture. The organism was considered a contaminant and was not studied further.

Proescher (13) in 1911 reported finding numerous bacilli in sections from the excised wound in his case. The organisms stained bluish with Giemsa solution in tissue fixed in bichloride and alcohol, but could not be found in sections hardened in formalin. They were straight or slightly curved, from 0.5 to 1.5 microns long, and showed irregular or bipolar staining. A guinea pig inoculated subcutaneously with a portion of the excised wound after a three weeks' incubation period developed enlarged lymph nodes followed by inflammatory skin lesions. Sections of the enlarged lymph nodes showed a great number of the same bacilli that were found at the site of the bite of the patient. The organism could not be cultivated.

Schottmüller (9) has recently reported a case of rat-bite fever from which he obtained an organism in pure growth in eight consecutive blood cultures taken during a period of two months. Colonies appeared well developed in agar plates on the second or third day. The organism was cultivated on blood agar, Loeffler's blood serum, milk agar, and in human blood serum, but failed to grow on other media. The colonies on solid media were discrete, colorless, and pin-head size. In human blood serum growth appeared as a whitish floccular sediment. Microscopical examination showed long, fine, twisted threads, some homogeneous, some fragmented, some branching. They often showed spindle- or ball-shaped swellings, and coccus-like forms developed. The organism was non-motile, stained with the ordinary stains, and was tinged bluish with Gram's method. Growth occurred only at incubator temperature. The organism was not pathogenic for rabbits or guinea pigs. Schottmüller considered the organism to be a streptothrix and has called it *Streptothrix muris ratti.*

From a case in its clinical manifestations closely resembling rat-bite fever following the bite of a South African squirrel he isolated a similar organism which he has designated *Streptothrix taraxeri cepapi.* Inoculation of a monkey proved the organism to be pathogenic for that animal.

Of the pathological anatomy of rat-bite fever but little is known. Only one autopsy by Miura (22) is published. Macroscopically there was increase of the cerebrospinal fluid and injection of the pial vessels of the brain and cord. No further pathological changes were recorded and no mention was made of microscopic examination. Histological study of the excised wound in Proescher's (13) case showed a mixed inflammatory and proliferative lesion. The corium was infiltrated with numerous endothelial cells, lymphocytes, and polynuclear leukocytes. Young connective tissue elements were noted, and the whole lesion was surrounded by a wall of dense, hyaline connective tissue. Examination of excised lymph nodes recorded in a few instances has shown only hyperplasia.

Further pathological changes can only be surmised from knowledge of the clinical features of the disease. The inflammatory process at the site of the wound, the lymphangitis, and adenitis are rarely if ever suppurative in character in the absence of secondary infection. Superficial or deep ulceration, vesicle formation, and gangrene may occur at the site of the bite. The extensive and often severe systemic symptoms presumably may be the result of a profound toxemia from the elaboration of a toxin at the site of the wound, or may be due to a general invasion of the body by the etiological agent. Whichever occurs, the effects are especially shown in the severe muscular pain, wide-spread exanthem, and profound disturbance of the nervous system.

There is meager evidence in the literature pointing to cardiac complications. In Millot-Carpentier's (4) case a systolic murmur was discovered at the base of the heart and over the carotid arteries late in the course of the disease, but there is no definite statement as to the patient's previous cardiac condition. Reece's (23) patient, who had previously been well, six months after recovery was unable to walk rapidly without becoming breathless, and causing violent palpitation of the heart. There is, however, no mention of a physical examination.

The respiratory system except for an occasional mild bronchitis seems to be equally exempt. Gastro-intestinal symptoms are rarely of importance. The liver and spleen are almost never enlarged. The rather frequent occurrence of nephritis as a severe and often fatal complication indicates that the kidneys may be severely damaged.

The following case of rat-bite fever, which terminated fatally and came to autopsy, has proved of considerable interest from the standpoint of etiology and pathology.

The patient entered the Peter Bent Brigham Hospital, May 15, 1915, in the service of Dr. Henry A. Christian, to whom I am indebted for the privilege of reporting the case. I wish here also to express my thanks for many courtesies from the pathological department of the hospital.

E. S. A. A white woman, age 67 years, born in New York, dry goods merchant. The patient was referred to the medical service of the Peter Bent Brigham Hospital on May 15, 1915, by Dr. William P. Bolles, of Roxbury, Mass., with the following history.

Complaint.—Rat-bite.

Family History.—Unimportant. Habits good.

Past History.—Measles in childhood. Malaria 20 years ago. She has had fibroids of the uterus for many years. Otherwise she has been very well. She has had no symptoms of respiratory, cardiac, gastro-intestinal, hepatic, or renal disease.

Present Illness.—Two weeks ago, while taking a rat out of a trap, the patient

was bitten on the tip of the right index finger. The wound was not severe and she paid no further attention to it than to wash it in hydrogen peroxide. Two days later the finger became reddened and red streaks appeared running up the forearm. The arm and right axilla became painful. The patient consulted a physician who opened and dressed the wound and prescribed phenacetine. After four days the inflammation and pain had entirely subsided and she resumed her ordinary course of life, feeling perfectly well until 10 hours before admission to the hospital. At that time she had a severe chill which lasted about thirty minutes, suffered severe pain in the legs, arms, and sides, felt very feverish, and was completely prostrated. She was nauseated, but did not vomit. No other symptoms were noted and her condition was unchanged at the time of admission.

Physical Examination.—The patient is a well developed and well-nourished woman, very drowsy, dull, and apathetic, evidently suffering from severe pain throughout the body, especially in the legs. There is no cough or dyspnea. Temperature 101.4° F.; pulse 108.

Skin is pale and dry; no exanthem. Eyes, ears, nose, mouth, and throat are negative. Neck is readily flexed. Thyroid not felt.

The right axillary lymph nodes are enlarged, not tender, non-fluctuant. No other nodes are palpable. Lungs are negative.

The heart's action is regular. Apex impulse is not felt. There are no thrills. Left border of dulness is 11 cm. to the left of the midsternal line in the 5th intercostal space; right border is 3 cm. to the right of the midsternal line in the 4th interspace; upper border is at the 3d rib. Heart sounds are muffled. At the apex a soft systolic murmur is heard, not widely transmitted. There is no diastolic murmur. A2 is slightly accentuated, P2 normal.

Pulses are equal, regular, synchronous, of small volume and moderated tension.

Blood pressure: systolic 140; diastolic 86.

In the right lower quadrant of the abdomen there is a large rounded firm tumor (fibroid). Otherwise abdominal examination is negative.

Liver and spleen are not palpable.

On the tip of the right index finger is a small linear scar. There is no inflammation about the scar and no evidence of lymphangitis. There is muscular tenderness in both arms and legs.

Reflexes are normal.

June 15. Blood: hemoglobin 92 per cent; red blood corpuscles 4,400,000; white blood corpuscles 22,100. Smear: neutrophils 74 per cent; small mononuclears 17 per cent; large mononuclears 8 per cent; eosinophils 1 per cent; basophils 0. The red blood corpuscles appear normal. No parasites.

Urine is amber, clear, acid; specific gravity 1.024; trace of albumin; sugar 0; acetone 0; diacetic acid 0; bile 0; sediment, many white blood corpuscles and finely granular casts. No blood.

Blood culture, see below.

Phenolsulphonephthalein test: 48 per cent excreted in 2 hours.

May 16. Pulse and temperature, see Text-fig. 1. Patient is apathetic and complains of severe muscular pains; has no appetite. A blotchy, bluish red, macular, and maculopapular exanthem has appeared on the arms, thorax, thighs, and knees. The macules vary from 2 to 6 mm. in diameter, are sharply marginated, slightly raised, fade on pressure, and are neither painful nor itchy.

May 17. Condition unchanged. Tendon reflexes of arms are exaggerated. Stained blood smears and fresh smears examined with dark-field illumination show no parasites or bacteria.

May 18. Exanthem has faded somewhat. General condition is unchanged. Stool is negative. Wassermann reaction is negative.

May 19. Blood culture, see below. Blood serum does not agglutinate *B. typhosus*. Urine contains a trace of albumin; sediment, few finely granular casts.

May 20. Patient appears very sick, is very apathetic. Exanthem has nearly disappeared. White blood corpuscles 19,300.

May 24. General condition is improved. White blood corpuscles 14,700.

May 25. Patient's temperature has fluctuated considerably during the past few days. She had a severe chill followed by a rise in temperature this evening.

May 27. Condition is steadily improving. Muscular pain and tenderness are slight. No exanthem is present.

May 28. Temperature normal. White blood corpuscles 17,400.

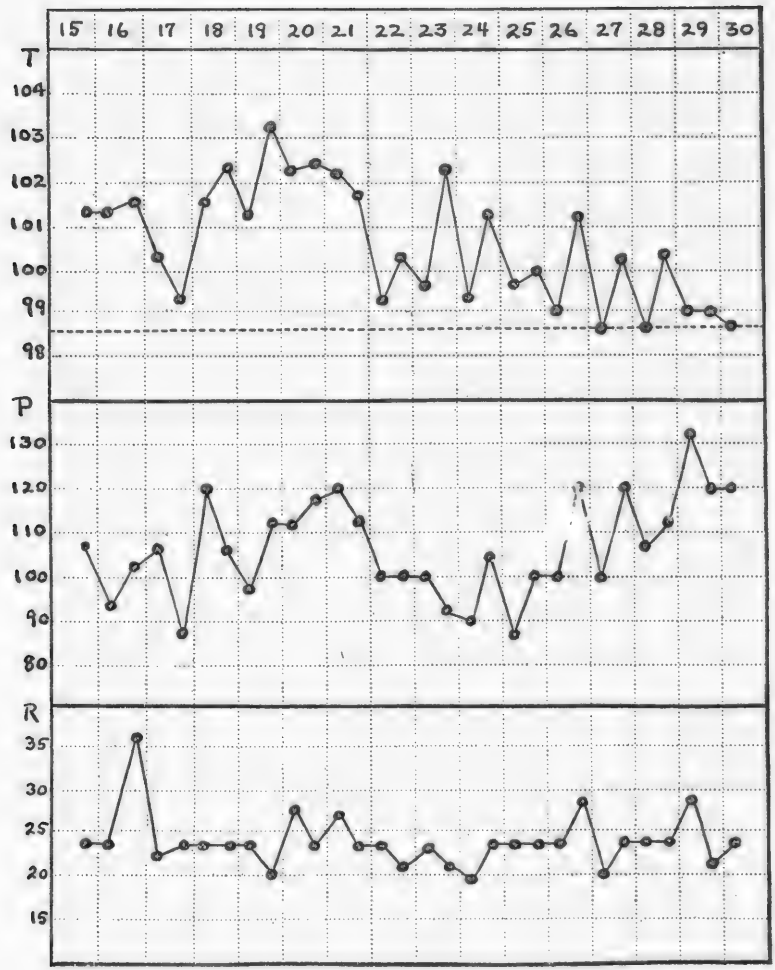
May 29. Patient was restless during the night. Pulse became rapid and weak. Patient appears very sick. Lungs are negative. The systolic murmur at the apex of the heart has become louder and harsher. A finely mottled, reddish, slightly indurated skin eruption 5 by 10 cm. has appeared on the outer aspect of the left knee.

May 30. Patient became gradually weaker and died at 7 a.m.

Autopsy Protocol.

The autopsy was done by Dr. James L. Stoddard 7 hours post mortem.

Fairly well nourished adult showing no pigmentation and no subcutaneous edema. Peritoneal cavity is free from fluid and signs of inflammation. There are several large uterine myomata. Pleural cavities contain a small amount of bloody fluid; no adhesions. Pericardial cavity contains about 5 cc. of clear yellow fluid; no adhesions. Heart (360 gm.) has a normal appearing musculature. Tricuspid, pulmonic, and aortic valves appear normal. On the posterior cusp of the mitral valve there is a large raised area made up of soft tissue, which was formed apparently under the endocardium. The area is 15 mm. in diameter and 7 mm. in height. In its center is a perfora-



TEXT-FIG. 1. Temperature chart of case of rat-bite fever (E. S. A.)

tion 4 mm. in diameter passing through the valve leaflet. The other cusps of the valve appear normal. Lungs show moderate congestion. Liver shows marked passive congestion. Spleen (200 gm.) shows a brownish red and pale mottling. On section it appears soft and edematous, the pulp scraping off easily. Two firm, pale, wedge-shaped areas are seen, with their bases at the surface of the spleen. Pancreas, gastro-intestinal tract, adrenals, and bladder appear normal. Kidneys are small (95 gm. each); surface is slightly granular; cortex 4 to 5 mm., pale gray, without distinct markings. Aorta shows marked sclerosis in the abdominal portion. Brain appears normal.

Microscopic Examination.—The myocardium shows areas of subacute myocarditis with complete disappearance of the muscle fibers, their place being taken by loose tissue filled with endothelial cells and leukocytes (Fig. 8). In the fibers elsewhere there is a high degree of fat vacuolation. Sections of the mitral valve through the vegetation (Fig. 11) show a central perforation surrounded by granulation tissue infiltrated with leukocytes. At the periphery there are masses of connective tissue, parts of which are hyalinized, parts thickly infiltrated with leukocytes, and parts necrotic. In the necrotic areas are masses of organisms appearing as long slender bacilli (Fig. 12). The muscle fibers adjacent to the lesion show all stages of necrosis.

The lung shows hemorrhage, edema, and passive congestion.

Sections of the liver show increased interlobular connective tissue, in many places thickly infiltrated with lymphocytes and polynuclear leukocytes (Fig. 9). In some places there is increased connective tissue extending within the lobules with slight infiltration with lymphocytes and polynuclear leukocytes. In these areas many of the liver cells are atrophic; elsewhere they appear normal.

The spleen shows marked hemorrhagic and necrotic areas. The adrenals show a perivascular exudate of lymphoid cells and moderate edema of the tissue throughout. The pancreas appears normal. Sections of the uterine tumor show an atrophic myoma.

The glomeruli of the kidneys are closely placed and large with considerable thickening of the capillary walls. There is an occasional completely sclerosed glomerulus. There is frequently an increased number of polynuclear cells in the capillaries. The interstitial tissue shows various stages from an acute to a healed process of uneven dis-

tribution. In many places there is an exudate chiefly of polynuclear cells extending in long streaks between the tubules (Fig. 10); in other places the exudate consists of lymphocytes and plasma cells, often situated about a glomerulus. In some places there is increased connective tissue. The tubular epithelium is granular, but shows little desquamation. The vessels show marked thickening of the intima and media with hyaline change. One section shows a small infarction.

Diagnosis.—Acute ulcerative endocarditis; subacute myocarditis; subacute interstitial hepatitis; subacute glomerular and interstitial nephritis; subacute perivascular exudate of adrenals; infarcts of spleen and kidney; congestion, hemorrhage, and edema of lungs; atrophic leiomyoma of uterus.

Bacteriological Data.—A blood culture was made on May 15, within 15 hours after the onset of systemic symptoms. 25 cc. of blood were drawn from the median basilic vein and distributed in dextrose agar and ascitic agar plates and deep tubes, plain and dextrose bouillon flasks and tubes, and Loeffler's blood serum slants. The cultures were incubated partly aerobically and partly anaerobically. On the third and fourth days after inoculation growth appeared in all the media, aerobic and anaerobic, except the dextrose agar plate and deep tube and the dextrose bouillon flask. These remained sterile. One colony appeared in the ascitic agar plate and three colonies in the ascitic agar deep tube. The colonies were grayish white, pin-head size, slightly irregular in outline. In bouillon the growth consisted of whitish flocculi which remained at the bottom of the tubes and flasks without clouding the media. In the Loeffler's blood serum slants the growth was most abundant, appearing as whitish flocculi in the blood and water of condensation at the bottom of the tubes.

A second blood culture was made on May 19. 20 cc. of blood were distributed in plain and ascitic bouillon, and Loeffler's blood serum tubes. No growth was observed in any of the media (seven days' incubation).

At autopsy, May 30, cultures of the heart's blood were made in 4 Loeffler's blood serum tubes and 3 bouillon flasks. After 48 hours' incubation all the blood serum tubes showed a moderate growth identical with that obtained in the first blood culture. One of the bouillon

flasks showed a staphylococcus which was considered a contaminant; the others remained sterile.

Cover-slip preparations from all the positive cultures showed a pure growth of a thin filamentous organism varying greatly in length. Many forms were homogeneous throughout their length, others fragmented giving the appearance of a chain of short bacilli. There were occasional forms containing deeply staining granules, and a few swollen spindle- and club-shaped forms. Here and there definitely branching forms were seen. These characteristics indicated that the organism was probably a streptothrix, a fact which further study has confirmed.

TABLE I.

Serum of patient E. S. A.	1:20	1:40	1:80	1:160	1:320	1:640	Salt solution.
2 hrs. at 37° C.	++	++	+	−	−	−	−
12 hrs. on ice	++	++	+	−	−	−	−
Normal human serum.	1:20	1:40	1:80	1:160	1:320	1:640	
2 hrs. at 37° C.	−	−	−	−	−	−	
12 hrs. on ice	−	−	−	−	−	−	

May 19. Numerous blood smears stained with Wright's stain and fresh dark-field preparations were carefully searched for parasites and organisms, but yielded negative results.

May 28. When the patient's temperature had fallen to normal, blood was drawn for agglutination tests with the organism isolated from the blood. 1 cc. of increasing dilutions of the patient's serum was mixed with 1 cc. of an 0.85 per cent salt solution suspension of the organism thoroughly shaken, in a series of small tubes and incubated for 2 hours at 37° C. in a water-bath. 0.85 per cent saline and the same dilutions of normal human serum were used as controls. Final readings were recorded after the tubes had stood in the ice-box for 12 hours. Positive agglutination occurred in dilutions of 1: 20, 1: 40, and 1: 80. The controls were negative. The results are shown in Table I (Fig. 7).

Agglutination tests were repeated in a similar manner after the

organism had been growing in subculture for three weeks, partial agglutination occurring in dilutions as high as 1:320 (Table II) after one hour's incubation.

TABLE II.

Serum of patient E. S. A.	1:20	1:40	1:80	1:160	1:320	1:640	Salt solution.
1 hr. at 37° C.....	C	C	C	++	+	–	–
12 hrs. on ice.....	C	C	C	++	+	–	–

C indicates complete agglutination and sedimentation.

Description of Organism.

Morphology.—The organism is a slender, filamentous, branching organism, growing in interwoven masses not manifesting a definite radial arrangement at the periphery of the colonies. In young cultures the filaments vary greatly in length, are curved, waving, or straight, show many true branching forms and stain homogeneously (Fig. 1). After 18 to 24 hours' incubation fragmentation of the filaments appears and branching forms rapidly disappear. The filaments now appear as chains of bacilli varying in length (Fig. 3). Staining is no longer homogeneous and numerous granular or beaded forms appear (Fig. 5). Occasional filaments become spindle-shaped, while others show ball-shaped, oval, or terminal club-shaped swellings, and chains of coccus-like forms appear (Fig. 2). Older cultures show marked fragmentation and breaking up of the filaments into rods (Fig. 4). The rods vary considerably in length, are straight or slightly curved, and are frequently beaded, resembling in many respects the pleomorphic bacilli. The chains of coccus-like forms become more numerous, and remaining masses of filamentous forms stain very faintly.

Staining Reactions.—The organism stains readily with methylene blue, gentian violet, carbol-fuchsin, pyronin, and Bismarck brown. It is Gram-negative and is neither acid- nor alcohol-fast.

Capsule.—No capsule formation could be demonstrated. The club-shaped forms do not show the characteristic sheath of actinomyces as described by Wright (24).

Spores.—No true endospores could be demonstrated.

Motility.—The organism is non-motile.

Oxygen Requirements.—The organism is a facultative anaerobe. Growth under anaerobic conditions is somewhat less abundant than under aerobic conditions.

Thermal Death Point.—The organism is killed by exposure to 60° C. for 10 minutes. Cultures containing chains of coccus-like forms were used in determining the thermal death point.

Viability.—Cultures kept at 37° C. die out after 5 to 8 days. On ice cultures remain viable 7 to 14 days.

Temperature Requirements.—The optimum temperature for growth is 37° C. Scanty growth appears after 4 days at room temperature.

Reaction of Media.—The optimum reaction for growth is between the neutral point and 2.0+. Growth does not occur in media of greater alkalinity than 1.5− or of greater acidity than 3.0+.

Pigment Formation.—The organism is non-chromogenic.

Cultural Characteristics.

Gelatin.—No growth.

Loeffler's Blood Serum.—After 24 hours at 37° C. a moderately abundant growth of discrete, whitish, pin-point, circular, sharply marginated, elevated, smooth, glistening, moist, non-mucoid colonies appears (Fig. 6). There is a whitish, flocculent growth in the water of condensation. The medium is not liquefied. After 48 hours' incubation colonies may show slight coalescence if closely aggregated, but they do not tend to increase in size after the first 24 hours' incubation.

Human Blood Agar.—After 24 hours at 37° C. a very scanty growth similar to that on Loeffler's blood serum appears.

Ascitic Agar.—When first isolated, the organism failed to grow on this medium. Growth was finally obtained, however, after the organism had been subcultured on Loeffler's blood serum for $2\frac{1}{2}$ months. Colonies show the same characteristics as on blood serum.

Ascitic Bouillon.—(Plain bouillon 4 parts, ascitic fluid 1 part.) After 18 to 24 hours at 37° C. a whitish flocculent growth appears at the bottom of the tube and to some extent along the sides (Fig. 6).

The medium remains clear and there is no surface pellicle. Growth ceases after 24 to 30 hours.

Plain and dextrose agar, plain and dextrose bouillon, ox bile, litmus serum water, potato, and litmus milk showed no growth after 7 days' incubation on repeated trials.

Fermentation.—No growth could be obtained in levulose, lactose, dextrose, mannite, maltose, saccharose, or dextrin litmus serum waters. A good growth was obtained in inulin, lactose, mannite, raffinose, saccharose, and salicin broths to which ascitic fluid had been added, 1:4, but there was no demonstrable change in reaction after 7 days' incubation.

Pathogenicity.—Guinea pigs, rabbits, and white rats were inoculated with blood from the patient, and with varying amounts of 24 hour cultures of the organism with the following results.

Four rabbits were inoculated, two intravenously and two intraperitoneally, May 19, 1915, each with 5 cc. of citrated blood from the patient. One rabbit died on May 26, 1915, of an intercurrent infection. The other three showed no evidence of disease. Autopsies, June 30, 1915, were negative.

Two white rats were inoculated intraperitoneally, May 19, 1915, each with 3 cc. of citrated blood from the patient. Neither showed any evidence of disease. Autopsies, June 22, 1915, were negative.

Six guinea pigs were inoculated, May 20, 1915, three subcutaneously, three intraperitoneally, with varying amounts of ascitic bouillon cultures. None of the animals showed any evidence of disease. Autopsies, June 22 and 29, 1915, were negative.

Two rabbits were inoculated intravenously, two intraperitoneally, and two subcutaneously, May 20, 1915, with varying dosages of 24 hour ascitic bouillon cultures. Five yielded entirely negative results during 6 weeks' observation and at autopsy. The protocol of the sixth experiment is given below.

Rabbit 6.—Weight 1,220 gm. Inoculated subcutaneously, May 20, 1915, with a salt solution suspension of the growth from 30 cc. of ascitic bouillon (24 hours' incubation).

May 25. Animal seems well. A firm nodule the size of a large pea has appeared beneath the skin at the site of inoculation.

June 1. Nodule is the size of a small marble, firm, non-fluctuant, apparently not tender.

June 30. The animal has shown no other evidence of disease than the subcutaneous tumor which has persisted. There has been a steady gain in weight. Animal killed.

Autopsy.—Weight 1,590 gm. In the subcutaneous tissue of the left flank at

the site of inoculation is a firm, slightly irregular, encapsulated tumor 1.2 cm. in diameter. On section it presents a yellowish white granular appearance with two small foci of caseation. The lymph nodes in the left axilla are enlarged, discrete, and firm. On section they present a yellowish white, homogeneous surface without suppuration or caseation. No other enlarged nodes were noted. Heart, lungs, liver, spleen, kidneys, adrenals, gastro-intestinal tract, brain, and spinal cord appear normal macroscopically.

Microscopic Examination.—The subcutaneous nodule shows a peripheral wall of dense fibrous tissue surrounding a reticulum of loose connective tissue in which are many large and small necrotic foci surrounded by fibroblasts, plasma, and endothelial cells. No giant cells are seen. No organisms are demonstrable. The lymph nodes show only hyperplasia. Sections of the lung, myocardium, liver, spleen, and kidneys appear normal.

Cultures made from the subcutaneous nodule and lymph nodes remained sterile.

Four white rats were injected with varying amounts of 24 hour ascitic bouillon cultures with the following results.

White Rat 1.—May 22, 1915. Intraperitoneal injection of a salt solution suspension of the growth from 75 cc. of ascitic bouillon (24 hours' incubation). The animal appeared sick for a few days following the injection, but by the end of a week had apparently entirely recovered. It remained well until June 28, 1915, when it was killed.

Autopsy.—There were numerous fibrous adhesions about the spleen, stomach. liver, and diaphragm. The mesenteric, axillary, and inguinal lymph nodes were enlarged. They appeared glistening on section and showed no areas of caseation. No other abnormalities were noted on gross examination.

Microscopic Examination.—The lymph nodes showed only hyperplasia. Sections of the spleen showed thickening of the capsule and general hyperplasia. Plasma cells and endothelial cells were numerous throughout and occasional giant cells were seen. There were no areas of caseation or necrosis. The lungs, myocardium, liver, and kidneys appeared normal.

Cultures from the spleen, heart's blood, and lymph nodes remained sterile.

White Rat 2.—May 22, 1915. Intraperitoneal injection with a salt solution suspension of the growth from a 30 cc. ascitic bouillon culture.

May 23, 1915. Animal found dead. Streptococcus septicemia.

White Rat 3.—May 22, 1915. Subcutaneous injection with a salt solution suspension of the growth from a 30 cc. ascitic bouillon culture. The animal remained well without local or general reaction until June 13, 1915, on which date a small area of induration appeared at the site of inoculation. The lesion gradually increased in size, and on June 16, 1915, broke down, discharging a small amount of seropurulent fluid. The animal appeared lively and showed no evidence of a general reaction. The cutaneous lesion which was 1 cm. in diameter became crusted over but showed no tendency to heal or to spread. No other lesions developed. The rat was killed on June 28, 1915.

Autopsy.—At the site of inoculation there was a well localized abscess 1 cm. in diameter which had discharged externally and was covered with a reddish brown crust. The lesion was situated in the subcutaneous tissue and thoroughly walled off by dense connective tissue. The center was filled with necrotic material and a purulent exudate. The axillary, inguinal, peritoneal, and thoracic lymph nodes were considerably enlarged. On section the nodes appeared glistening grayish white, and showed no areas of caseation. No other gross abnormalities were noted.

Microscopic Examination.—The lymph nodes showed only hyperplasia and the presence of numerous plasma cells and endothelial leukocytes. Sections of the abscess showed a mass of necrotic material infiltrated at the margin with fibroblasts and plasma cells and surrounded by dense connective tissue. No giant cells were seen. Sections of the spleen, kidney, liver, lung, and myocardium appeared normal.

Smears from the abscess showed numerous rod-shaped and coccus-like organisms and a few filamentous forms. Cultures from the abscess showed an abundant growth in pure culture of the organism with which the rat was inoculated. Cultures of the heart's blood and lymph nodes remained sterile.

White Rat 4.—July 1, 1915. Subcutaneous injection of a salt solution suspension of the streptothrix isolated from Rat 3. The growth from 50 cc. of a 24 hour ascitic bouillon culture was used. The animal remained well and showed no local or general reaction. It was killed Aug. 11, 1915. Autopsy showed one enlarged axillary lymph node which on microscopic examination showed moderate hyperplasia. There were no other abnormalities.

In summary, the organism is very slightly pathogenic for rabbits and white rats, producing in some cases a local inflammatory and proliferative reaction at the site of inoculation, and general lymph node hyperplasia. It is not pathogenic for guinea pigs. Passage through one rat did not increase its pathogenicity.

DISCUSSION.

The diagnosis of rat-bite fever in the case described above seems thoroughly justified by the clinical picture which the patient presented. The history of a rat-bite, latent incubation period, non-suppurative lymphangitis, high fever ushered in by a severe chill, severe generalized muscular pain, stuporous condition, and the bluish red macular exanthem were quite typical of the recognized symptom-complex of the disease.

The isolation of an organism in pure culture from the blood during life and at autopsy and the development of an agglutinin in the

patient's blood serum for that organism leaves little question as to its causative relationship to the disease in this case. The presence of a morphologically similar organism in the sections of the mitral vegetation in the absence of other demonstrable organisms is strong presumptive evidence that the same organism was the cause of the endocarditis.

Study of the organism has shown it to be a true branching, filamentous organism, showing fragmentation of the mycelial filaments, granule formation, and the development of chains of coccus-like forms. It is Gram-negative and not acid-fast. In ascitic bouillon it grows as a flocculent sediment without clouding the media, and on solid media it grows in discrete colonies. These characteristics place the organism in the group of filamentous fungi, the Hyphomycetes. The nomenclature of both species and genera in this group is still somewhat confused, different authors using several generic names,—Nocardia, Actinomyces, Streptothrix, Discomyces, Oospora. The weight of opinion and common usage, however, favor the adoption of the name Streptothrix for the genus, and it seems best to classify the organism described above as one of the Streptothrices.

Claypole (25), Foulerton (26), and Musgrave, Clegg, and Polk (27) have done much to clarify our knowledge of this group of organisms. The latter authors give the following generic characters for the Streptothrices: "Branching, filamentous organisms which develop into colonies made up of the organisms and 'transformation products.' The terminal hyphæ may or may not be radially placed on the surface of the colony and they may or may not develop 'clubs.' The group in general take Gram's stain and several members show acid-fast properties in a varying degree." Claypole states that the members of the group are alike in the following characteristics: "When grown in bouillon, the medium always remains clear. The growth on solid media is discrete. . . . . They are all gram positive, all have granule formation, either in mycelium or its products, as shown in chain sporulation, and, finally, branching organisms and thread-like forms are seen more or less frequently in all species."

The organism isolated in this case shows all the typical features of a streptothrix with the exception that it is Gram-negative, a variation which does not seem sufficiently important to exclude it from the genus.

The question of species is a more difficult one. The literature contains numerous more or less complete descriptions of organisms isolated from cases of streptothricosis. Musgrave, Clegg, and Polk (27) recognize six well established species: *S. actinomyces*, Boestrom (1890); *S. actinomyces*, Wolff and Israel (1891), Wright (1905); *S. nocardii; S. eppingeri; S. maduræ*, Vincent; *S. capræ*, Silberschmidt. The organism isolated in our case is definitely distinct from any of these, and differs from any streptothrix hitherto described with the exception of that isolated by Schottmüller in his case of rat-bite fever. Although Schottmüller does not give a complete description of his organism, it agrees in most particulars with the organism isolated in our case, the only difference being that Schottmüller's organism was "tinged bluish" with Gram's stain and it did not grow at room temperature. In spite of these slight differences it seems probable that the organisms are identical, and for that reason advisable to accept his name for the organism,—*Streptothrix muris ratti.*

Two features of interest in our case in addition to the relation of the organism to rat-bite fever are isolation of a streptothrix in pure culture from the blood during life, and the presence of an ulcerative endocarditis caused by a streptothrix. Cases of generalized streptothricosis resembling pyemia are not infrequently recorded in the literature, but the only other instance of a streptothrix septicemia in which the organism was isolated by blood culture is Schottmüller's case of rat-bite fever. Cases of streptothrix endocarditis are equally rare. Naunyn (28) in 1888 found in endocarditis vegetations of a case of chorea a branching organism which was identified as a cladothrix or leptothrix by Zopf. Pappenheimer and Satchwell (29) isolated a filamentous organism from the blood in a case of endocarditis and demonstrated the same organism in sections of the aortic vegetations. They considered their organism to be a cladothrix. No other instances of endocarditis caused by a filamentous microorganism are recorded in the available literature.

SUMMARY.

The similarity in the cases of rat-bite fever recorded in the literature establishes it as a definite clinical entity. The same symptomatology occurs in cases from Asia, Europe, and America. The greater fre-

quency of the disease in Japan than elsewhere is probably due to the housing conditions and habits of the people resulting in the more frequent occurrence of rat-bites. It does not seem necessary to consider that cases occurring in Europe and America are due to the bites of rats that have been imported from Japan.

The clinical picture and course of the disease indicate that it is infectious in origin. Until Schottmüller's case appeared in 1914, the etiology had been undiscovered. He isolated from his case in eight consecutive blood cultures a streptothrix which he has designated *Streptothrix muris ratti*. His work has been confirmed by the isolation of an identical streptothrix from the blood during life and at autopsy in the case here reported. Further confirmation of the etiological relationship of this organism to the infection in our patient is found in the production of powerful agglutinins for the organism in the blood serum of this case and in the demonstration of the organism in the vegetation on the mitral valve. It is not unreasonable to suppose that Proescher (13) observed the same organism in the sections of the excised wound in his case. Although it is fully realized that Koch's postulates have not been fulfilled in the absence of successful animal experimentation, nevertheless the accumulated evidence here presented leaves little reason to doubt that the specific cause of rat-bite fever is *Streptothrix muris ratti*.

The pathology of rat-bite fever has hitherto been largely a matter of surmise. One autopsy only has been recorded in the literature (Miura (22)), and nothing abnormal was noted other than injection of the pial vessels. The autopsy in the case here reported has proved of considerable interest in the extent and character of the lesions found. A streptothrix septicemia with the localization of the organism in the mitral valve producing an acute ulcerative endocarditis is the most striking feature of the case. The infarcts of the spleen and kidney are a natural sequence of the endocarditis. The subacute lesions of the myocardium, liver, adrenal, and kidneys, glomerular and interstitial, are all of a similar nature, consisting of areas infiltrated with leukocytes, lymphocytes, plasma, and endothelial cells with varying degrees of degeneration of the normal cells of the affected area. In no instance has the presence of the streptothrix in these lesions been demonstrated, and it is not unreasonable to assume that they are toxic in origin.

The data here presented may be correlated with the clinical features of rat-bite fever to give us a clear understanding of the course and nature of the disease. The patient is inoculated by the bite of a rat with *Streptothrix muris ratti*. After a variable incubation period a non-suppurative inflammatory reaction occurs at the site of the wound with extension to the neighboring lymphatics and lymph nodes. Invasion of the blood stream follows, accompanied by the onset of severe toxic symptoms. Clinically the nervous system and frequently the kidneys seem to be especially involved. That the myocardium, liver, and adrenals may also suffer is shown by the autopsy findings in the case reported above. Ulcerative endocarditis is probably a rare occurrence. In the majority of cases after a more or less prolonged course, the disease terminates spontaneously and so may be considered a self-limited infection. This is presumably brought about by the development in the body of a protective mechanism against the streptothrix. That such a process does occur is evidenced by the demonstration of agglutinins in our case. Whether a permanent immunity is acquired after one attack of rat-bite fever is not known. No instances of a second infection are recorded in the literature.

Although rat-bite fever varies somewhat in its symptomatology in individual cases, the picture is sufficiently characteristic to make the diagnosis not a difficult matter. The history of a rat-bite, latent incubation period with subsequent non-suppurative inflammatory reaction of the wound, lymphangitis, and enlarged lymph nodes, severe chill at onset, high fever of the relapsing type, intense muscular pain and nervous symptoms, and the characteristic bluish red exanthem, present a symptom-complex not easily overlooked. The disease is frequently complicated by a severe nephritis, and prolonged cases develop a high grade of anemia and cachexia. In the case here reported ulcerative endocarditis occurred.

In the large majority of cases the prognosis is favorable for a successful termination. The patients, however, are often incapacitated for a considerable period of time The mortality is about 10 per cent, death usually occurring in the first febrile period apparently from a profound toxemia, or at a later stage due to the development of a severe nephritis.

Until recently treatment has been entirely symptomatic and has been of little avail in altering the course of the disease. Miyake has found immediate treatment of the wound by cauterization or with carbolic acid highly efficient as a prophylactic measure. Hata (30) in 1912 introduced salvarsan therapy and reported eight cases so treated, seven of which showed marked and rapid improvement. One case was apparently unaffected. Two of the cases receiving only small doses had a subsequent relapse. Surveyor (31) and Dalal (18) also have reported success with salvarsan injections. It is to be hoped that further experience with this method of treatment will yield equally favorable results.

CONCLUSIONS.

1. Rat-bite fever is a specific infectious disease following the bite of a rat. It occurs in Asia, Europe, and America.

2. The etiological organism is *Streptothrix muris ratti*, first described by Schottmüller in 1914. His observation is confirmed by the isolation of an identical streptothrix in the case here reported.

3. Invasion of the blood stream by *Streptothrix muris ratti* occurs in rat-bite fever.

4. The case here reported developed a powerful agglutinin for *Streptothrix muris ratti*.

5. Pathological changes occur in the myocardium, kidneys, liver, and adrenals showing areas of degeneration and infiltration with polynuclear leukocytes, lymphocytes, plasma cells, and endothelial cells.

6. Ulcerative endocarditis may occur in rat-bite fever and be caused by the *Streptothrix muris ratti*.

BIBLIOGRAPHY.

1. Miyake, H., *Mitt a.d. Grenzgeb. d. Med. u. Chir.*, 1900, v, 231.
2. Wilcox, W., *Am. Jour. Med. Sc.*, 1840, xxvi, 245.
3. Horder, T. J., *Quart. Jour. Med.*, 1909–10, iii, 121.
4. Millot-Carpentier, *L'union méd.*, 1884, xxxviii, 1069.
5. De Micas, *Arch. méd. de Toulouse*, 1895, i, 170.
6. Curtillet and Lombart, *Jour. de méd. int. Paris*, 1912, xvi, 335.
7. Peña y Maya, F., *Siglo Med.*, 1885, xxxii, 117.
8. Frugoni, C., *Riforma med.*, 1911, xxvii, 1298.

9. Schottmüller, H., *Dermat. Wchnschr.*, 1914, lviii, Supplement, 77.
10. Ogata, *Mitt. a. d. med. Fakult. d. k. Univ. z. Tokyo*, 1909, viii, 287.
11. Atkinson, A. G., *Med. Chron., Manchester*, 1912–13, series 4, lvii, 1.
12. Gouget, A., *Presse méd.*, 1912, xx, 186.
13. Proescher, F., *Internat. Clin.*, 1911, series 21, iv, 77.
14. Crohn, B. B., *Arch. Int. Med.*, 1915, xv, 1014.
15. Farquhar, O. C., *Cincinnati Lancet and Obs.*, 1869, xii, 650.
16. Nixon, J. H., *Brit. Med. Jour.*, 1914, ii, 629.
17. Dick, M. I., and Rutherford, W. J., *Brit. Med. Jour.*, 1913, ii, 1581.
18. Dalal, A. K., *Practitioner*, 1914, xcii, 449.
19. Ogata, M., *Mitt. a. d. med. Fakult. d. k. Univ. z. Tokyo*, 1911, ix, 343.
20. Ogata, *Mitt. a. d. med. Fakult. d. k. Univ. z. Tokyo*, 1913–14, xi, 179.
21. Middleton, G. S., *Lancet*, 1910, i, 1618.
22. Miura, M., and Toriyama, N., *Ztschr. d. med. Gesellsch. z. Tokyo*, 1897, xi, 1059.
23. Reece, M., *Med. Exam.*, 1872, xiii, 51.
24. Wright, J. H., *Jour. Med. Research*, 1904–05, xiii, 349.
25. Claypole, E. J., *Jour. Exper. Med.*, 1913, xvii, 99.
26. Foulerton, A. G. R., *Lancet*, 1910, i, 552, 626, 769; 1913, i, 381.
27. Musgrave, W. E., Clegg, M. T., and Polk, M., *Philippine Jour. Sc.*, 1908, iii, B, 447.
28. Naunyn, B., Mittheilungen aus der medizinischen Klinik zu Königsberg i. Pr., Leipzig, 1888, 296.
29. Pappenheimer, A. M., and Satchwell, H. H., *Jour. Infect. Dis.*, 1907, iv, 617.
30. Hata, S., *München med. Wchnschr.*, 1912, lix, 854.
31. Surveyor, N. F., *Lancet*, 1913, ii, 1764.

EXPLANATION OF PLATES.

PLATE 8.

FIG. 1. Stained preparation of *Streptothrix muris ratti* from a 15 hour Loeffler's blood serum culture, showing branching, homogeneously stained filaments and the non-radial arrangement at the margin of the colony.

FIG. 2. *Streptothrix muris ratti* from a 24 hour Loeffler's blood serum culture, showing irregularly swollen filaments and beginning formation of coccus-like chains.

PLATE 9.

FIG. 3. Fragmentation of mycelial threads appearing as chains of bacilli (24 hour culture).

FIG. 4. Complete fragmentation into rod-like forms (30 hour culture).

FIG. 5. Fragmented forms showing beading and terminal swellings.

PLATE 10.

FIG. 6. Twenty-four hour culture of *Streptothrix muris ratti* on Loeffler's blood serum and in ascitic bouillon.

FIG. 7. Agglutination of *Streptothrix muris ratti* by patient's serum. Dilutions 1 : 20, 1 : 40, 1 : 80, and control (Table I).

PLATE 11.

FIG. 8. Section of myocardium from patient.

FIG. 9. Section of liver from patient.

PLATE 12.

FIG. 10. Section of kidney from patient.

PLATE 13.

FIG. 11. Section of mitral vegetation showing necrotic material, infiltration with polymorphonuclear leukocytes, and masses of organisms.

PLATE 14.

FIG. 12. Section of mitral vegetation showing clumps of organisms. The rod-like form of the organisms is indefinite in the single focal plane represented in the microphotograph, but can easily be demonstrated in microscopic study of the actual section.

ANTIBLASTIC IMMUNITY.

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As a result of the study of the natural or acquired resistance of animals against infection with living microorganisms, many previously unknown properties of the cells and fluids of the body have been discovered. The value of these properties as resistance factors is dependent upon the degree of antagonism which they manifest to invasion with living foreign elements. On the other hand, virulence of bacteria may be said to be proportional to the strength of defence which they possess against such antagonistic forces. In the face of such opposition, however, the bacterium must not only maintain its life, but in order to increase must be able to obtain a proper food supply. The mechanism by means of which bacteria extract from living tissue the substances essential to their nutrition is not well understood, nor can we say to what extent the animal body opposes this appropriation of its own supplies. It is in the hope of throwing light upon this obscure problem that the following study has been undertaken.

The microorganism used was the pneumococcus, not because it is especially suitable, but because many of the materials necessary for the experiments were easily obtainable. The hypothesis upon which our efforts are based is that bacteria do not assimilate all of their foodstuffs in the condition in which they exist in the medium of their environment, but that certain changes must be effected before absorption occurs. The preparation of nutritional substances may take place upon the surface of the bacterial cell or in its immediate neighborhood, and this function, when carried on within the substance of a living animal, may be opposed by certain inhibitory forces. The interaction of these phenomena may play an important part in resistance and immunity to infection.

By immunization of the horse to pneumococcus, a serum can be prepared which protects susceptible animals against many times the fatal dose of virulent pneumococci. A complete understanding of the mode of action of this serum is still lacking. However, the fact has been observed and confirmed that antipneumococcus serum possesses neither bactericidal nor bacteriolytic action *in vitro*. Indeed, pneumococcus is known to grow in considerable concentration of its homologous immune serum. In the following experiment (Table I) is shown the rate of growth of pneumoccoccus in homologous and in heterologous antipneumococcus serum and in normal horse serum. The amount of growth was determined by the plate method.

TABLE I.

Experiment I. Inhibition of Growth of Pneumococcus by Antipneumococcus Serum.

Serum 0.1 cc.	Culture 0.00001 cc.	Immediately.	After 3 hrs.
Antipn. serum Type I.....	Pn. I	230 colonies.	216 colonies.
" " " II.....	" I	200 "	630 "
Normal.....	" I	200 "	1,400 "

This experiment shows clearly that a marked inhibition of the growth of pneumococcus occurs in antipneumococcus serum, as compared with normal horse serum. The inhibitory effect of homologous immune serum is greater than that of heterologous immune serum; in fact, in the former serum no increase in the number of pneumococci has taken place in three hours. The inhibition of growth may be explained in four ways: first, that agglutination occurs in immune serum, and that the failure to increase is apparent only; second, that the formation of threads is responsible for the apparent inhibition; third, that we are dealing with the bacterial lag of freshly planted cultures; and fourth, that active interference with the growth phenomena of the organisms has occurred. That the first two reasons are entirely responsible for the inhibition of growth is disproven by the fact that a marked delay in development occurs in heterologous immune serum in which no agglutination whatever takes place and in which thread formation is not more extensive than in normal serum. The latent period that marks the growth of freshly planted

cultures of bacteria cannot explain the inhibition, inasmuch as this would affect the culture in normal serum as well as that in immune serum, since the same culture of pneumococcus was used for seeding and the conditions of cultivation were similar in each tube. We are forced, then, to conclude that these various phenomena do not entirely explain the inhibition of growth, and that such inhibition as occurs is largely dependent upon some property of immune serum which adversely affects the circumstances of multiplication. The inhibitory influence of immune serum is manifest only for a relatively short period of time, for if plates are made at the end of twenty-four hours, the pneumococcus is found to have overcome the inhibition, and innumerable colonies develop from comparable amounts of all the cultures.

Carbohydrate and protein form the main food supply of most bacteria. In our hypothesis we have suggested that these substances may not be absorbed unchanged and that some form of preparation may occur at the surface of the bacterial cell. If immune serum is added to the medium in which the cell is growing, the changes necessary for the development of food substances appropriate for assimilation would be subject to the influence of any inhibitory bodies present in the immune serum. In the following experiments evidence is brought that antipneumococcus serum possesses the power to inhibit both the splitting of protein and the fermentation of sugar by pneumococcus. The organisms were grown in serum broth for twenty-four hours. The amount of protein splitting has been estimated by the increase in amino nitrogen determined by the method of Van Slyke.

The following experiment is one of a series showing the degree of protein splitting that occurs when pneumococci are grown in broth containing normal horse serum, and the effect of substituting antipneumococcus serum for normal serum in the mixtures. The estimations of the increase in amino nitrogen were made after twenty-four hours' incubation at 37° C., a period at which marked growth of the pneumococcus had occurred both in the tubes containing normal serum, and in those containing immune serum. In those tubes in which the pneumococcus has grown in its homologous serum, agglutination has occurred. There has been, however, no agglutination either in normal serum or in heterologous immune serum.

TABLE II.

Experiment II. Inhibition of Digestion of Protein by Pneumococcus with Antipneumococcus Serum.

				Increase in amino nitrogen per cc.
Pn.	Type	I + 1 cc.	normal horse serum + 4 cc. broth.....	0.21 cc. nitrogen.
"	"	I " 1 "	antipn. serum Type I + 4 cc. broth.....	0.02 " "
"	"	I " 1 "	" " " II " 4 " "	0.06 " "
"	"	II " 1 "	normal horse serum " 4 " "	0.21 " "
"	"	II " 1 "	antipn. serum Type I " 4 " "	0.14 " "
"	"	II " 1 "	" " " II " 4 " "	0.06 " "

The figures of this experiment (Table II) make it strikingly evident that the addition of antipneumococcus serum to growing cultures of pneumococcus markedly diminishes, in some instances almost to the point of extinction, the production of amino-acids by the organism. The formation of amino-acids in mixtures of broth and normal horse serum has been found generally equivalent to the amount found in broth alone. Certain other normal animal sera have, however, been observed which inhibit the process in varying degrees. Inhibition by homologous immune serum is most complete, although, as in the experiment showing inhibition of growth of pneumococcus, heterologous immune serum possesses considerable inhibitory power. Whether the increase in amino-acid is due to endogenous or exogenous metabolism cannot, of course, be definitely determined. We believe, however, that, in the utilization of protein, the pneumococcus effects a splitting of the protein before absorption, and that the increase in amino-acid represents the excess of protein split, over that used up in the process of growth.

Carbohydrate, as is well known, is an important part of the food supply of most bacteria, and the great variety of sugars that many organisms ferment is extraordinary. That such a splitting of sugars is in some way associated with the nutrition of microorganisms seems a logical assumption. We have found that the addition of antipneumococcus serum to cultures of pneumococcus containing such sugars as glucose, saccharose, lactose, and inulin inhibits in varying degrees the fermentation of the sugars by the organism. Inhibition of fermentation of inulin is most complete, and an example of this inhibition is given in Experiment III (Table III). Pneumococcus, when added to serum water media containing inulin, and litmus as

an indicator, ferments the inulin which results in acidification of the medium and coagulation of the serum.

TABLE III.

Experiment III.

	24 hrs.	48 hrs.	72 hrs.	5 days.
Pn. Type I + inulin.....	++	++	++	++
" " I " antipn. serum Type I + inulin.	−	−	−	−
" " I " " " " II " "	Sl. ac.	Ac.	++	++

++ indicates complete acidification and coagulation; + indicates acid and incomplete coagulation; ± indicates acid and beginning coagulation; V. sl. ac. indicates very slight acidification; Sl. ac. indicates slight acidification; Ac. indicates slight acidification and no coagulation; − indicates no acidification or coagulation.

Experiment III shows that the addition of homologous immune serum to a culture of pneumococcus in inulin completely suspends fermentation of the inulin. Heterologous immune serum delays the reaction, but does not entirely inhibit it. Fermentation of the sugars more actively attacked by pneumococcus, such as glucose, lactose, and saccharose, is not inhibited to the same degree as that of inulin. Determination of the rate of production of acid in cultures containing such easily fermentable sugars shows that, in the early hours of growth, the formation of acid is markedly delayed by the presence of immune serum. After twenty-four hours, however, the acid concentration may reach the same degree in all tubes and, in general, represents the grade of acidity at which pneumococcus ceases to grow. The splitting of carbohydrates by bacteria probably occurs at the surface of the bacterial cell, as is thought to be the case in fermentation of sugar by yeast, and the anti-enzymotic forces of immune serum in all probability exert their antagonistic action at this point.

A study of human blood serum obtained at intervals during the course of an attack of lobar pneumonia shows that bodies having an anti-enzymotic action similar to that of immune serum develop during the period of recovery from the disease. The tests were made in the same manner as those in which an artificially prepared immune

serum was used. The two following experiments are typical of the results obtained (Tables IV and V).

TABLE IV.

Experiment IV. Inhibition of Digestion of Protein by Pneumococcus with Human Serum in Lobar Pneumonia. Infection with Pneumococcus Type II.

	Increase in amino nitrogen per cc.
Pn. Type II + 2 cc. broth + 0.5 cc. serum, 5 days before crisis.....	0.16 cc. nitrogen.
" " II " 2 " " " 0.5 " " at crisis.....	0.02 " "
" " II " 2 " " " 0.5 " " 9 days after crisis.....	0.02 " "

TABLE V.

Experiment V. Inhibition of Fermentation of Inulin by Pneumococcus with Human Serum in Lobar Pneumonia. Infection with Pneumococcus Type I.

	24 hrs.	48 hrs.	72 hrs.	5 days.
Inulin + Pn. Type I.....	++	++	++	++
" " " " I + serum 8 days before crisis...	±	++	++	++
" " " " I " " 6 " " " " ...	+	++	++	++
" " " " I " " just after crisis.....	−	V.sl.ac.	Sl.ac.	Ac.
" " " " I " " 7 days " "	Sl.ac.	Ac.	±	±

These experiments show clearly that at the crisis of lobar pneumonia substances appear in the serum either for the first time, or in greatly increased amount, which have the power of inhibiting the proteolytic and glycolytic activities of the pneumococcus. The period of development of these substances corresponds in time with that of other immune bodies which have been recognized in the serum of individuals with lobar pneumonia.

DISCUSSION.

The series of experiments presented in this paper demonstrate the following facts. Antipneumococcus serum possesses the power of inhibiting for a certain period of time the multiplication of pneumococci. In conjunction with this capacity, it has also the power of inhibiting in varying degree the proteolytic and glycolytic functions of pneumococci. This power is present to a limited extent in the

sera of certain normal animals and absent in others, and in human sera during the course of an attack of lobar pneumonia it appears for the first time or increases markedly at the critical period of the disease. From these facts we are led to assume that retardation of growth is, in part at least, dependent upon inhibition of metabolic function. The observation that immune serum possesses in high degree the powers described, suggests that these properties play an important part in resistance and immunity to infection with pneumococcus. Investigators have demonstrated previously that certain other immune sera possess analogous qualities; such as the inhibition of pigment production by *Bacillus pyocyaneus* (1), the liquefaction of gelatin by *Staphylococcus pyogenes aureus* (2), and the formation of methemoglobin by pneumococcus (3). We have chosen the term "antiblastic immunity" as descriptive of this phenomenon, in order to indicate that the forces at work are antagonistic to the growth activities of the organism. Ascoli (4) coined the term several years ago (*βλαστειν*, to grow). From his studies in anthrax immunity, he was led to suppose that the latter was in part dependent upon the inhibition of formation by *Bacillus anthracis* of a capsule which is a prerequisite for its successful development in the animal body, and he ascribed to anti-anthrax serum an antiblastic action, directed against the metabolic activity of this organism. A concrete interpretation of this phenomenon as applied to the growth of pneumococcus and the inhibitory influence of immune serum is as follows: Pneumococcus, in order to grow, must obtain a sufficient supply of protein and carbohydrate; these substances are furnished by the environmental medium, but probably require, to render them suitable for absorption, preliminary preparation in the nature of digestion. This change is effected at the surface of the bacterial cell and the integrity of this digestive zone is essential to the growth of the bacterium. Anti-enzymotic bodies such as have been demonstrated in immune serum act at the point of contact of the cell with its environment, and influence in an unfavorable manner the nutritional processes there carried on, and the consequence of such action is retardation or inhibition of growth. It is possible that capsule formation represents on the part of the organism an attempt to protect the function of the digestive zone. Should the foregoing prove

to be a correct explanation of the phenomenon observed, considerable light would be thrown on the obscure mechanism by means of which parasitic bacteria establish themselves in animal tissues, and on the forces mobilized by the animal body in opposition to such invasion.

CONCLUSIONS.

1. Antipneumococcus serum possesses the power of inhibiting for a certain period of time the multiplication of pneumococci.

2. It also has the capacity of inhibiting the proteolytic and glycolytic functions of pneumococci.

3. This power is acquired for the first time or appears in increased amounts in human serum at the time of crisis in lobar pneumonia.

4. The retardation of bacterial growth is thought to be dependent upon the inhibition of metabolic function due to the presence of anti-enzymotic substances in antipneumococcus serum. To this phenomenon we have applied the term antiblastic immunity.

BIBLIOGRAPHY.

1. Ghéorghiewsky, *Ann. de l' Inst. Pasteur*, 1899, xiii, 298.
2. von Dungern, *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1898, xxiv, 710.
3. Cole, R., personal communication.
4. Ascoli, A., *Centralbl. f. Bakteriol., 1te Abt., Orig.*, 1908, xlvi, 178.

ATHEROMA AND OTHER LESIONS PRODUCED IN RABBITS BY CHOLESTEROL FEEDING.

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PLATES 15 TO 19.

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Ignatovski (1) in 1908 published the results of his experiments on the effect of animal food on the rabbit. By feeding milk and egg yolk to these animals he produced various lesions, the most noteworthy of which were atheroma of the aorta, cirrhosis of the liver, and enlargement of the adrenals. In the following year Starokadomsky and Ssobolew (2), also by feeding rabbits on milk and egg, confirmed the results of Ignatovski in so far as the production of an atheroma of the aorta was concerned. They also obtained lesions in the innominate artery, carotids, and subclavians. Ignatovski ascribed his results to animal proteids. Several workers had previously described lesions produced in rabbits by animal food (d'Amato (3) lesions of the aorta, and Garnier and Simon (4) lesions of the liver), but these lesions differed essentially from those produced by Ignatovski and the latter's results attracted particular attention on account of the similarity of the aortic lesions as described by him to human atheroma. Stuckey (5) also confirmed Ignatovski's results. He afterward attempted to determine what part of the food given was responsible for the lesions, trying (6) milk, meat juice, egg albumin, and egg yolk, with the result that lesions as described by Ignatovski were produced only by the egg yolk. Later (7) he used different animal and plant fats, fish liver oil, beef fat, and sunflower-seed oil with negative results. He did, however, produce lesions of the aorta, apparently identical with those described by Ignatovski, by feeding rabbits on brain.

Chalatow (8) studied the livers of Stuckey's rabbits and reported characteristic changes in the livers of those which had been fed on egg and brain. In these he found an abundant infiltration of the parenchyma with doubly refracting fat.

Wesselkin (9) fed rabbits on egg and milk and others on lecithin and milk. In the former he obtained an abundant deposit in the liver, aorta, and spleen of lipoid substances which he considered mainly cholesterol esters. In the lecithin-fed rabbits also he found in the same organs a deposit of lipoids which, from their microchemical reactions, he believed to be phosphatids. Because, however, no pathological changes followed the deposit of the latter in the organs, while marked changes were produced by the cholesterol esters, he concluded that the injury was to be ascribed to the cholesterol of the egg yolk.

Anitschkow and Chalatow (10) fed pure cholesterol and produced characteristic lesions, and later also Wacker and Hueck (11). Weltmann and Biach (12), however, though they produced a cirrhosis of the liver, were unsuccessful in obtaining aortic lesions; and van Leersum (13) who fed two rabbits on eggs and two on pure cholesterol found a suspicious lesion in but one of the four. This was an egg-fed rabbit which had been under treatment for a shorter period than any of the other three. These authors are unable to explain their lack of results. Van Leersum states that spontaneous atheroma occurs in about 3 per cent of rabbits, and apparently believes the one lesion observed in his rabbits is to be so explained.

Anitschkow and Chalatow (10, 14) produced similar lesions in guinea pigs by egg feeding, with an abundant deposit of anisotropic fat in the organs, but failed entirely in rats. Adler (15) found aortic lesions in each of two dogs fed on cholesterol, and he also found lesions in two of three dogs receiving cottonseed oil.

Aschoff (16) and Anitschkow (17) believe that other factors, especially an increase in blood pressure, are important in the production of the aortic lesions. The latter believes that the formation of such lesions in these rabbits is facilitated by increasing the blood pressure in the aorta by constricting its lumen in the abdominal portion, by suspending the animals according to the method of Klotz (18), or by the use of adrenalin.

For the purpose of studying the lesions produced by cholesterol-containing food, nine rabbits were fed on egg yolk and six on pure cholesterol. The egg yolk and the cholesterol, the latter dissolved in cottonseed oil by heating, usually in the proportion of 0.5 gm. to 10 cc. of oil, were mixed with the rabbits' daily ration of crushed barley. On an occasional day greens were given with the barley without the addition of egg or cholesterol. One rabbit, as a control, received large doses of cottonseed oil without cholesterol. This rabbit was fed on oil over a much longer period than any of the rabbits receiving cholesterol, and none of the latter received as much oil in the course of their feeding as did the control in a similar period.

Smears and frozen sections of the fresh tissue were examined for doubly refracting fat under the polarizing microscope. Frozen sections of the tissues fixed in Orth's fluid were stained with Sudan III and by Van Gieson's method; paraffin sections with Van Gieson's method, hematoxylin and eosin, and in some cases with Weigert's elastic tissue stain and Mallory's connective tissue stain.

EXPERIMENTAL.

Rabbit 1.—Control. Received 1,216 cc. of cottonseed oil in 248 days. For short periods no oil was given; the remainder of the time it took from 3 to 23 cc. of oil per day. At autopsy no gross lesions were found. The left adrenal was larger than normal, the right very small, measuring but 2 mm. in diameter. The combined weight was normal,—0.28 gm. On microscopical examination the aorta, liver, spleen, and kidneys were found normal. The liver contained a very small amount of isotropic fat in the peripheries of the lobules. No anisotropic fat was found except in the adrenals.

Rabbit 2.—Received 0.01 gm. of cholesterol per day with the exception of an occasional day. Took 0.29 gm. of cholesterol in 33 days.

Gross Appearances.—Aorta, pulmonary artery, spleen, liver, and kidneys normal. Adrenals normal size; combined weight, 0.37 gm.

No anisotropic fat was found in the aorta, spleen, liver, or kidneys.

Rabbit 3.—Received 0.01 gm. of cholesterol per day with the exception of an occasional day. Took 0.5 gm. in 55 days.

Gross Appearances.—Aorta, pulmonary artery, spleen, liver, and kidneys normal. Adrenals normal size; combined weight, 0.35 gm.

No anisotropic fat was found in the aorta, liver, spleen, or kidneys.

Rabbit 4.—Received 0.05 gm. of cholesterol per day with the exception of an occasional day. Took 3.8 gm. in 84 days.

Gross Appearances.—Aorta, pulmonary artery, spleen, liver, and kidneys normal. Adrenals slightly enlarged; combined weight, 0.41 gm.

No anisotropic fat was found in the aorta, liver, or kidneys; a very small amount in the spleen.

Rabbit 5.—Received 1 egg yolk per day with the exception of an occasional period of a few days. Took 49 yolks in 78 days.

Gross Appearances.—Pronounced intimal lesions in aorta and pulmonary artery. Spleen about three times normal size. Liver slightly enlarged, surface smooth, cuts with somewhat increased resistance, shows pronounced fine deep yellow mottling. Adrenals greatly enlarged; combined weight, 1.35 gm. Kidneys show a few small scars on the surface; in the medulla, mainly in the outer zone, but extending into the inner zone are very numerous yellow opaque streaks and nodules. Large amount of anisotropic fat in the aortic lesions, liver, spleen, and kidneys.

Rabbit 6.—Received from 0.1 gm. to 0.5 gm. of cholesterol per day with the exception of a few days. Took 16.4 gm. of cholesterol in 83 days.

Gross Appearances.—Moderate intimal lesions in the aorta, confined to arch and thoracic portion, and in pulmonary artery. Spleen about normal size. Liver normal size, surface smooth, cuts with somewhat increased resistance, shows pronounced fine yellow mottling. Adrenals slightly enlarged; combined weight, 0.4 gm. Kidneys show a few small scars on the surface, a few opaque yellow lesions in the outer zone of medulla. Large amount of anisotropic fat in the aortic lesions, liver, spleen, and kidneys.

Rabbit 7.—Received 1 egg yolk per day with the exception of an occasional period of a few days. Took 58 yolks in 85 days.

Gross Appearances.—Surface of aorta dull, but no definite lesions apparent here or in the pulmonary artery. Spleen normal. Liver slightly enlarged and yellow. Adrenals enlarged; combined weight, 0.69 gm. Kidneys show no scars on surface, a few very faint yellow lines in outer zone of medulla. Moderate amount of anisotropic fat in intima of aorta, large amount in liver, very small amount in spleen and kidneys.

Rabbit 8.—Received 1 egg yolk per day with the exception of an occasional day. Took 26 yolks in 30 days.

Gross Appearances.—Pronounced intimal lesions in aorta, most numerous in arch and thoracic portion, but present throughout, also in pulmonary artery. Spleen normal in size. Liver shows pronounced fine yellow mottling, otherwise normal. Adrenals slightly enlarged (not weighed). Kidneys show no scars on surface; medulla is a diffuse yellowish color with a few definite yellow streaks in the outer zone.

Anisotropic fat in aortic lesions, liver, spleen, and kidneys.

Rabbit 9.—Received 1 egg yolk per day with the exception of an occasional period of a few days. Took 47 yolks in 98 days.

Gross Appearances.—Very marked intimal lesions throughout aorta, extending into large vessels of neck, renal arteries, and iliacs; also in pulmonary artery. Spleen moderately enlarged. Liver appears normal. Adrenals enlarged; combined weight, 0.56 gm. Kidney shows numerous scars on surface, and in outer zone of medulla very thickly set and pronounced yellow nodular lesions, extending as fine yellow lines into inner zone.

Large amount of anisotropic fat in aortic lesions and kidney, moderate amount in liver and spleen.

Rabbit 10.—Received 0.5 gm. of cholesterol per day with the exception of an occasional day. Took 14.5 gm. in 36 days.

Gross Appearances.—Moderate intimal lesions in aorta, mostly in arch and thoracic portion, a few in abdominal portion; also in pulmonary artery. Spleen moderately enlarged. Liver shows pronounced fine yellow mottling. Adrenals slightly enlarged (not weighed). Kidneys show a few small scars on the surface, numerous yellow lesions in outer zone of medulla extending into inner zone. Large amount of anisotropic fat in aortic lesions, liver, spleen, and kidneys.

Rabbit 11.—Received 1 egg yolk per day with the exception of an occasional interval of a few days. Took 54 yolks in 103 days.

Gross Appearances.—Very marked intimal lesions throughout aorta, most marked in arch and thoracic portion, also in iliacs; marked lesions in pulmonary artery. Spleen moderately enlarged. Liver shows pronounced yellow mottling. Adrenals enlarged; combined weight, 0.58 gm. Kidneys show numerous scars on surface; in outer zone of medulla numerous very pronounced yellow lesions, extending also into inner zone. Large amount of anisotropic fat in aortic lesions, liver, and kidneys; small amount in spleen.

Rabbit 12.—Received ½ to 1 egg yolk per day with the exception of an occasional interval. Took 68 yolks in 138 days.

Gross Appearances.—Marked lesions in the arch of the aorta, smaller lesions in the thoracic and abdominal portions; moderate lesions in the pulmonary artery. Spleen moderately enlarged. Liver about normal size, surface smooth, cuts with somewhat increased resistance, shows pronounced yellow mottling. Adrenals enlarged; combined weight, 0.72 gm. Kidneys appear normal. Large amount of anisotropic fat in aortic lesions and liver, a moderate amount in spleen, very little in kidneys.

Rabbit 13.—Received 1 egg yolk per day with the exception of an occasional day. Took 27 yolks in 34 days.

Gross Appearances.—Moderate intimal lesions in aorta and pulmonary artery. Spleen about normal size. Liver appears normal. Adrenals enlarged; combined weight, 0.55 gm. Kidneys show numerous irregular depressed scars on surface, in outer zone of medulla a moderate number of yellow opaque streaks. Anisotropic fat abundant in aortic lesions and kidney, small amount in liver and spleen.

Rabbit 14.—Received 0.1 to 0.3 gm. of cholesterol per day with the exception of an occasional interval of a few days. Took 8.6 gm. of cholesterol in 97 days.

Gross Appearances.—Very pronounced intimal lesions from valves to exits of renal arteries, a few small lesions below; marked lesions in pulmonary artery. Spleen normal size. Liver shows slight yellow mottling, otherwise normal. Adrenals greatly enlarged; combined weight, 1.4 gm. Kidneys show no scars on surface, in outer zone of medulla a slight diffuse yellow color. Large amount of anisotropic fat in aortic lesions and liver, moderate amount in kidney, very little in spleen.

Rabbit 15.—Received 1 egg yolk per day with the exception of an occasional day. Took 65 yolks in 79 days.

Gross Appearances.—Marked intimal lesions in aorta and pulmonary artery. Spleen about twice normal size. Liver shows pronounced yellow mottling, otherwise normal. Adrenals enlarged, combined weight, 0.84 gm. Kidneys appear normal. Large amount of anisotropic fat in aortic lesions, liver, and spleen; only a very few fine droplets in kidney.

Rabbit 16.—Received ¼ to ½ egg yolk per day with the exception of an occasional interval of a few days. Took 32½ yolks in 134 days.

Gross Appearances.—Slight intimal lesions in arch and upper thoracic portion of aorta, none elsewhere; slight lesions in pulmonary artery. Spleen moderately enlarged. Liver shows slight fine yellow mottling. Adrenals enlarged; combined weight, 0.62 gm. Kidneys appear normal. Large amount of anisotropic fat in aortic lesions and liver, a moderate amount in the kidney, little in the spleen.

The previously mentioned authors agree in the main in their descriptions of the aortic lesions. To summarize very briefly, they describe the production of yellowish white raised plaques and stripes

mainly in the arch of the aorta. Microscopically these lesions were mainly intimal and showed a pronounced thickening due to a collection of cells, prominent among which were very large cells filled with doubly refracting fat. Similar fat was also found lying outside the cells. In the later lesions considerable production of elastic fibers was observed with some degeneration of the large cells. A small amount of doubly refracting fat was present in the upper layers of the media. The adventitia was not affected.

The preceding brief summary of our experiments shows that in eleven of the fifteen rabbits lesions of both aorta and pulmonary artery were visible in the gross. One additional rabbit, No. 7, in which lesions were not evident macroscopically, showed very numerous characteristic but relatively slight lesions microscopically. The gross lesions varied greatly in number, size, and severity, but were always quite evident (Fig. 1). They were invariably most marked in the arch and thoracic portion and in some of the less severe cases were found only here. In several of the latter the lesions were especially prominent about the exits of the large vessels arising from the arch or of the intercostals; in others a tendency to this localization was not apparent. The lesions consisted of small (in the early cases a fraction of a mm.) raised, yellowish white, rather sharply defined spots, or less frequently of fine streaks lengthwise of the vessel. In more marked cases larger irregular areas, several mm. in diameter, were seen evidently formed by the confluence of many smaller spots. These in cases involved a large portion of the arch and thoracic portion, leaving only comparatively small irregular areas of normal intima. The lesions of the pulmonary artery were similar to those of the aorta, but in most cases not so pronounced.

The intima of the rabbit's aorta is normally very thin, consisting of a single layer of endothelium and possibly an occasional fibroblast lying on the internal elastic layer of the media. The early lesions produced by cholesterol feeding consist of a cellular thickening of the intima, most of the cells present showing small droplets of doubly refracting fat. This fat is seen in spindle cells, a few drops appearing at either end of the nucleus, and in large, swollen, round or somewhat irregular cells which are loaded with the fat. The nuclei of these

cells vary, some being small, round, and deeply staining, others larger, round or oval, and vesicular.

There has been considerable discussion as to the nature of these fat-containing cells, as there has been concerning the similar cells seen in human atheroma. Large cells similar in appearance and loaded with doubly refracting fat are seen in various localities in these rabbits, in the bone marrow, in the spleen both in the pulp and free in the venous sinuses, and in the kidney in the capillaries of the medulla and in interstitial lesions to be described. These large fat-containing cells in the situations mentioned are apparently of endothelial type (histiocytes of Kiyono), and it would seem probable that the similar cells in the aorta are of the same nature. In marked cases, however, the fat is also seen in cells which are evidently fibroblasts, as in the epicardial connective tissue, about the bronchi in the lungs, and in the capsule and trabeculæ of the spleen. In these cells the fat is present as a few small droplets at either end of the nucleus. The fat content of the cells in these situations is always small and the large swollen cells are not seen. It is believed that in the intimal lesions of the aorta also fibroblasts take some part in the absorption of the fat.

Anitschkow (19) calls attention to the similarity in the distribution of the doubly refracting fat to that of the dye in vital staining. In the latter process also fibroblasts show a few granules of the dye at either end of the nucleus, while endothelial cells are very deeply stained. Tschaschin (20) has made an extensive study of these vitally staining cells, and also Aschoff and Kiyono (21). Tschaschin shows that these cells correspond to the resting wandering cells of Maximow, and a further source, especially in inflammation, he believes to be the hematogenous lymphocytes. These vitally staining cells he terms polyblasts. Anitschkow (19) believes that in part at least the fat-containing cells in the aortic lesions of cholesterol-fed rabbits are of this origin. He applies to them the term cholesterin ester-phagocytes. Aschoff and Kiyono (21) introduced the term histiocytes for these vitally staining cells, and believe that they are ultimately derived from the endothelium and certain cells of the reticulum of the blood-forming organs.

The fat-containing cells, especially in the early lesions, vary considerably in size, form, and fat content, and it seems possible to follow gradations from the spindle cells with a few fat droplets to the large cells filled with fat. As previously stated, it is believed that

the fibroblasts here as elsewhere play a part in taking up the fat, but from the appearance of gradations mentioned it does not necessarily follow that fibroblasts swell up to form the large cells described.

By this collection of cells an enormous thickening of the intima is produced, frequently to nearly twice the thickness of the media. In the earliest lesions observed the greater part of the fat was intracellular. A collection of fine fat droplets, apparently extracellular, is, however, sometimes seen lying along the inner surface of the internal layer of elastic tissue as described by Anitschkow. As the lesions progress there is a pronounced proliferation of fibroblasts and an abundant formation of collagen and fine elastic fibers, especially in the upper portion of the lesion. The large collection of fat-containing cells in the lower portion breaks down, and areas of degeneration are formed in the lower intima with an abundant deposit of cholesterol crystals, and in some cases calcification, capped by fibrous tissue (Fig. 2). Calcification was observed in the aortic lesions in four rabbits, Nos. 9, 11, 12, and 16. Two rabbits, 11 and 14, received calcium lactate with some of their feedings; Rabbit 11 about 0.5 gm. in each of 7 feedings, and Rabbit 14 a similar amount in 31 feedings. Since No. 11 showed a deposit of calcium, while No. 14 did not, and since three rabbits which received no additional calcium in their food showed such a deposit, it is evident that the addition of calcium to the rabbits' regular diet is not necessary to obtain calcification, and it would seem that the process is not facilitated by such addition.

Although the lesion is mainly intimal, it is not so limited. Even in the earlier cases some fat may be seen in the media, mostly in the upper third, but occasionally as deep as the middle, both in cells and extracellular between the layers of elastic tissue. In the more marked lesions the upper layers of elastic tissue are raised by the collection of fat and fat-containing cells in this region, and the same degeneration as described in the intima is seen here.

In Rabbits 12 and 16, which were fed longer than any of the other rabbits but with smaller doses, the aortic lesions differed somewhat from those in the other rabbits. While the intimal lesions were of the same character with many large fat-filled cells, the intimal thickening was not nearly as pronounced, fibrosis was slight, and areas of

degeneration with the deposit of cholesterol were not found. The media, however, was more markedly affected than in the rabbits receiving larger doses for a shorter period. Underlying the intimal lesions the media showed many nodular areas and longitudinal streaks, between the elastic layers, of granular fatty material with a considerable deposit of calcium salts. These were present in places, in Rabbit 12, as far as the middle of the media.

Wesselkin (9) noted lesions at the beginning of the pulmonary artery similar to those in the aorta. These lesions were present in eleven of fifteen rabbits in this series. In eight of these rabbits lesions were present not only at the beginning of the vessel but in its branches in the lungs. These lesions consisted of pronounced nodular intimal thickenings containing many large cells filled with fat (Fig. 3). They were very similar to those in the aorta. Degeneration with the deposit of cholesterol crystals, fibrosis, and calcification were present in some of the more pronounced lesions in the larger branches (Figs. 4 and 5), but were not as marked as in the aorta. The media was affected to a varying extent. In the severe cases some of the small branches showed similar intimal thickenings, occasionally so pronounced as apparently completely to obliterate the lumen.

The splenic lesions have been described by Ignatovski (22, 23) Anitschkow (24, 19), and Wesselkin (9). The findings in our series confirm in the main the observations of these authors. The spleen in Rabbit 5 was about three times normal size, in No. 15 somewhat over twice normal size. In five others there was moderate enlargement. Doubly refracting fat was present in all spleens except those of Nos. 2 and 3, but varied considerably in amount. The fat was contained in large cells in the pulp, similar large cells free in the venous sinuses, and in the endothelial lining cells of the sinuses. The cells free in the venous sinuses also contained considerable yellow granular pigment. The Malpighian bodies showed very little fat but an occasional large fat-containing cell was seen. Fat was sometimes present in small amount in the endothelium of the arteries, and their walls sometimes gave a diffuse orange stain with Sudan III. No intimal thickenings were observed. In the severe cases the fibroblasts in the capsule and trabeculæ showed a few fat droplets at either end of the nucleus.

Enlargement of the adrenals has been noted by several of the previously mentioned investigators and also by Rothschild (25), who reports experiments on the relationship of the adrenals to cholesterol metabolism and hypercholesterinemia. Enlargement of the adrenals appears to be a consistent finding, having been present in all rabbits except Nos. 2 and 3. In Rabbits 5 and 14 these organs were about four times the normal size. They were of a uniform, almost white, color except for a very small pinkish medullary portion. Beyond the excessive anisotropic fat content no lesions were observed.

The liver lesions have been especially studied by Chalatow (26). He obtained somewhat different results in rabbits fed on pure cholesterol from those in rabbits

fed on egg yolk. In the former the doubly refracting fat was mainly in the peripheries of the lobules and there was marked increase of connective tissue here, which, however, did not invade the lobule. In one case the fat was present in both the peripheries and centers of the lobules and there was an increase of connective tissue in both places. In the rabbits fed on egg the doubly refracting fat was in the centers of the lobules. In these rabbits there was an increase of periportal connective tissue, which, however, was more diffuse and invaded the lobules. He describes a marked proliferation of the bile ducts with subsequent regeneration of the liver cells from them. In the older cases the liver surface was irregular, but the organ was not reduced in size.

In our series the livers, with a few exceptions noted in the summary, were of a deep yellow color; they were of normal size or slightly enlarged, and the surface was always smooth. Doubly refracting fat was present in all cases except Nos. 2, 3, and 4; in most in very large amount. The author cannot confirm Chalatow's observation as to the different localization of the fat in the rabbits fed on cholesterol and those on egg yolk. No differences were noted between the two series either as to location of fat or production of connective tissue. A very large portion of the fat was situated in the central portions of the lobules. It was contained both in the Kupffer cells and in the parenchymal cells. Some fat was also present in the periportal spaces, contained in fibroblasts and large endothelial cells. There was a considerable degeneration of the liver cells about the central veins, even in the earlier cases. Small focal areas of degeneration were frequent in the neighborhood of the central veins or midway in the lobules. Cirrhosis was present in six rabbits, Nos. 5, 6, 7, 10, 11, and 12. In Nos. 5 and 12 there was a pronounced formation of fibrous tissue; in the other four the process was slight. In all these cases there was a formation of fibrous tissue about the portal spaces and also in the centers of the lobules. In the former region the connective tissue encroached on the periphery of the lobule, and small islands of liver cells were isolated by the fibrous overgrowth. Fibrous tissue was formed about the central veins and extended radially outward in fine strands into the lobule (Fig. 6). The small areas of focal degeneration mentioned become fibrous in the more pronounced cases.

Chalatow (26) believes that the proliferation of interlobular connective tissue occurs only in part as a reactive phenomenon, as is the case in atrophic liver

cirrhosis where proliferating connective tissue takes the place of liver cells in the peripheries of the lobules. In great part it proliferates, stimulated by the newformed epithelium of bile ducts, to form a stroma for them.

It is true that the degenerated cells in the centers of the lobules and in the areas of focal degeneration are ultimately replaced by fibrous tissue. In the peripheries of the lobules, however, little if any degeneration of the liver cells is apparent, as a primary process at least, and in the author's rabbits the strands of cells in the connective tissue have the appearance of included liver cells rather than proliferating bile ducts. It is also worthy of note in this connection that the fat is present in the periportal areas in endothelial cells and fibroblasts. It would seem, therefore, that the fibrosis occurs as a result of the irritative action of the anisotropic fat on the connective tissue itself and its consequent proliferation rather than as a secondary reaction to the destruction of liver cells or for the purpose of forming a stroma for proliferating bile ducts, as believed by Chalatow.

Spontaneous cirrhosis is a common lesion in rabbits as pointed out by Ophüls (27), and it is possible that the periportal increase of connective tissue is in part to be thus accounted for. It is believed, however, that the intralobular formation of connective tissue, as seen in these rabbits, is not observed as a spontaneous lesion and there seems no room for doubt that this at least is due to the cholesterol feeding.

Little mention is made of the kidneys by any of the writers on the subject. Chalatow (28) says: "As the connective tissue phagocytes are scattered through all organs one can find in a rich circulation of cholesterol in the blood of rabbits, an isolated cell in nearly all organs which contains anisotropic fat. . . . . So one can, for example in the kidney in the interstitial cells, oftenest in the region of the collecting tubules, sometimes observe single cell elements of the type of connective tissue cells which are infiltrated with anisotropic fat." This author considers the process of anisotropic fatty infiltration of organs under two divisions: an infiltration of the stroma, in which the fat is present in phagocytic cells, which he terms xanthomatosis, and an infiltration of the parenchymatous cells, which he terms myelinosis. For the latter process he considers a preexisting deposit of isotropic fat to be necessary to bind the cholesterol, and he says (28): "Under the usual conditions a myelinosis of the kidney does not occur experimentally. And it is apparent why this is so, since in order to produce a myelinosis of the kidney it is necessary first to produce a deposit of isotropic fat in the kidney epithelium of longer or shorter duration. . . . . This, however, is very difficult to produce experimentally." Weltmann and Biach (12) had previously, with this same idea in mind, fed rabbits on cholesterol and in the course of the feeding injected uranium nitrate intraperitoneally. They thus obtained a deposit of anisotropic fat in the tubular epithelium.

The gross appearance of the kidneys in our series has been briefly given in the outline of the experiments. The kidneys of eight rabbits showed characteristic macroscopic lesions which, however, varied

considerably in severity. These consisted of opaque yellowish white stripes and nodules up to 1 mm. in breadth situated in the outer zone of the medulla, standing out sharply from the pink of the surrounding kidney tissue. In the more pronounced cases, Rabbits 5, 9, and 11, these were large and so numerous as to cover a large portion of the outer zone (Fig. 7). In these more severe cases fine yellow lines extended from the large lesions in the outer zone into the deeper portions of the medulla. In Rabbits 6, 7, 8, 10, and 13, lesions of the same character were present, but smaller and fewer in number.

These yellow lesions are due to a localized deposit of anisotropic fat. The kidneys show a small amount of doubly refracting fat in the tubular epithelium, which, however, is not confined to any particular region, but is found in the tubules of both cortex and medulla. Whether or not a neutral fatty degeneration preexisted would be difficult to decide. The striking lesion microscopically in these kidneys, however, and that to which the nodular lesions seen in the gross are due is not that of the tubules but of the interstitial tissue. Accepting the classification of Chalatow, it is a xanthomatosis rather than a myelinosis. The fat is contained for the most part in endothelial cells, both those of the capillaries and those of the lymph spaces, but especially the latter. The fat is seen in the endothelial cells of the capillaries to some extent throughout the kidneys, less abundant, however, in the capillaries of the glomeruli and elsewhere in the cortex than in the medulla. The fat so situated is small in amount, a few fine drops being seen in the cells at either end of the nucleus. Three rabbits, Nos. 12, 14, and 16, in addition to the eight mentioned above, showed this diffuse distribution of fat but without the nodular lesions seen in the latter. These nodular deposits are confined to the outer zone of the medulla in the less pronounced cases; in the more severe they extend into the deeper medulla but never outward into the cortex. In these areas the endothelial cells of the lymph spaces appear as large round or irregular cells filled with doubly refracting fat, very similar in appearance to the cells described in the intima of the aorta (Fig. 8). In more pronounced cases there are large collections of these swollen anisotropic fat-filled cells. In two rabbits, 9 and 11, many of these lesions show in their centers large areas of degeneration of the fat-containing cells, with an abun-

dant deposit of cholesterol crystals (Fig. 9). In No. 11 there is also some deposit of calcium in these degenerated areas. The tubules are for the most part destroyed, but an occasional collapsed tubule is seen passing through the lesions. Some of the capillaries in the deeper medulla, underlying these lesions, contain large numbers of round swollen cells filled with fat (Fig. 10). In only one rabbit, No. 11, a single nodular lesion of the intima of one of the larger vessels in the cortex was found, similar to those described in the vessels of the lung. The kidney lesions, therefore, do not appear to be due to lesions of the large vessels.

That some relationship exists between scars in these kidneys and the cholesterol lesions is evident. It will be noted from the outline that of the eight rabbits whose kidneys showed cholesterol lesions in the gross, six showed also scars on the surface of the kidneys, and that none of the kidneys of the other rabbits showed scars. These scars are similar in appearance to those seen in the so called spontaneous nephritis of rabbits, appearing in the gross as small, slightly depressed, purplish areas, and microscopically as cellular or fibrous interstitial lesions with collapse or disappearance of the tubules. In these kidneys nearly every scar shows at its base, in the outer zone of the medulla, a cholesterol lesion as described above. These collections of large fat-containing cells are never seen in the scars in the cortex but always at their base in the medulla. Rarely a scar is seen in the cortex at whose base no cholesterol lesion is apparent in the sections as cut; and occasionally a cholesterol lesion is seen with no overlying scar in the cortex. In Rabbit 8 scars extending through the cortex are not seen either in the gross or microscopically. There are, however, a considerable number of cholesterol lesions in the outer zone of the medulla. These are early; that is, they are made up of large fat-containing cells without areas of degeneration. Between the large cells are seen a few connective tissue fibrils. In Rabbit 7 there are no scars in the cortex. In the outer zone of the medulla, situated between the tubules, are a few very small longitudinal cellular scars. These show none of the large fat-containing cells described, but many spindle cells, apparently fibroblasts, contain a few fat droplets.

From the appearance of the tissue immediately surrounding the

older lesions it seems apparent that the cholesterol deposited in these areas in the kidney produces some proliferation of fibroblasts as it does in the aorta and in the liver. Whether, however, the cholesterol lesions are responsible for the scars in the cortex or whether the scars preexisted, is an important but difficult matter to decide from these few rabbits. If the scars preexisted the percentage of spontaneous nephritis in these rabbits was very high. On the other hand, it is to be noticed that a few scars were seen at whose base no cholesterol lesions were found, that in one case, No. 13, the scarring was very pronounced in a rabbit which had been fed for but 34 days, and that two rabbits, Nos. 12 and 15, whose kidneys showed no scars, and who, from a comparison with other rabbits, would seem to have been fed a sufficient amount of cholesterol and for a sufficient length of time to produce lesions, nevertheless showed none. These facts are in favor of the view that the scars preexisted and that a preexistent scar or perhaps merely a nodular infiltration of endothelial cells is necessary for the formation of these lesions. If this is so it remains to be explained why the large anisotropic fat-containing cells do not appear and cholesterol lesions are not formed in scars or portions of scars lying in the cortex. Small scars in the cortex about the glomeruli and elsewhere show no lesions, and scars extending through the cortex show cholesterol lesions only at their base in the medulla.

Considering the results as a whole it is found that the feeding of cholesterol-containing food to rabbits produces a widely distributed deposit of doubly refracting fat. This process is primarily one of infiltration, not of degeneration. For the production of such a deposit exceedingly large doses of cholesterol are necessary relative to the rabbit's body weight. This is shown by the results in Rabbits 2, 3, and 4, which were purposely given small doses to test this point. In none of these rabbits were any lesions found or any deposit of doubly refracting fat, except in the adrenals, which in No. 4 were slightly enlarged, and in the spleen of No. 4 which showed a very few large cells in the pulp containing the fat. Where this fat is deposited in large amounts, characteristic lesions are secondarily produced, notably an atheroma of the aorta, lesions of other vessels, mainly intimal, especially of the branches of the pulmonary artery, a cirrhosis of the liver, and lesions in the kidney as described.

Aschoff (16), Anitschkow (24, 17), Zinserling (29), and others discuss the possible relationship between the aortic lesion and human atheroma. Such a discussion would necessarily be at the present time largely theoretical and beyond the province of this paper. It seems, however, worthy of note and of possible importance for human pathology that in the rabbit an experimentally produced deposit of doubly refracting fat in the aorta finally results in a lesion practically identical with that seen in human atheroma. On the other hand, these lesions in the aorta are constantly associated with conditions in the spleen, liver, and pulmonary vessels which are not commonly found in human atheroma, but which seem to be an important part of the general picture of an overloading of the animal body with cholesterol. These additional manifestations could not be eliminated by feeding smaller doses. The rabbit is evidently an animal unable to cope with large doses of cholesterol, although small doses are comparatively innocuous.

SUMMARY.

The feeding of egg yolk or pure cholesterol to rabbits produces an abundant deposit of anisotropic fat in various organs. From this deposit characteristic lesions secondarily result in certain organs. Prominent among these lesions is an atheroma of the aorta very similar in the gross and histologically to the human lesion. Lesions of other vessels are also produced, conspicuous among which are those of the branches of the pulmonary artery. There is a large deposit of anisotropic fat in the liver which produces a cirrhosis. Enlargement of the adrenals occurs, probably due to the storage of an excessive amount of anisotropic fat. In a certain proportion of rabbits conspicuous lesions are produced in the kidneys consisting of nodular deposits of anisotropic fat in the medullary portion, the fat being contained for the most part in endothelial cells and fibroblasts in the interstitial tissue. Later softening occurs in these areas as in the aorta; the cells break down; there is an abundant deposit of cholesterol crystals, some calcification, and a proliferation of connective tissue. Scars frequently extend outward from these lesions through the cortex, but the nodular deposits of anisotropic fat are

never seen in the cortex. It is impossible to determine definitely from these experiments whether or not these deposits are dependent on preexisting interstitial lesions.

In conclusion I wish to acknowledge my indebtedness to Dr. Ophüls for his advice and assistance in this work, and to Dr. Jean Oliver for suggestions and for his assistance in taking the photomicrographs.

BIBLIOGRAPHY.

1. Ignatovski, A. I., *Izviest Imp. Voyenno-Med. Akad., S. Peterb.*, 1908, xvi, 154.
2. Starokadomsky, L. M., and Ssobolew, L. W., *Frankfurt. Ztschr. f. Path.*, 1909, iii, 912.
3. d'Amato, L., *Virchows Arch. f. path. Anat.*, 1908, cxcii, 86.
4. Garnier, M., and Simon, L.-G., *Compt. rend. Soc. de biol.*, 1907, lxiii, 250.
5. Stuckey, N. W., *Centralbl. f. allg. Path. u. path. Anat.*, 1910, xxi, 668.
6. Stuckey, N. W., *Centralbl. f. allg. Path. u. path. Anat.*, 1911, xxii, 379.
7. Stuckey, N. W., *Centralbl. f. allg. Path. u. path. Anat.*, 1912, xxiii, 910.
8. Chalatow, S. S., *Virchows Arch. f. path. Anat.*, 1912, ccvii, 452.
9. Wesselkin, N. W., *Virchows Arch. f. path. Anat.*, 1913, ccxii, 225.
10. Anitschkow, N., and Chalatow, S., *Centralbl. f. allg. Path. u. path. Anat.*, 1913, xxiv, 1.
11. Wacker, L., and Hueck, W., *München. med. Wchnschr.*, 1913, lx, 2097.
12. Weltmann, O., and Biach, P., *Ztschr. f. exper. Path. u. Therap.*, 1913, xiv, 367.
13. van Leersum, E. C., *Virchows Arch. f. path. Anat.*, 1914, ccxvii, 452.
14. Anitschkow, N., *Beitr. z. path. Anat. u. z. allg. Path.*, 1913, lvi, 379.
15. Adler, I., *Jour. Exper. Med.*, 1914, xx, 93.
16. Aschoff, L., *Beihefte z. med. Klin.*, 1914, x, No. 1.
17. Anitschkow, N., *Beitr. z. path. Anat. u. z. allg. Path.*, 1914, lix, 306.
18. Klotz, O., *Centralbl. f. allg. Path. u. path. Anat.*, 1908, xix, 535.
19. Anitschkow, *Beitr. z. path. Anat. u. z. allg. Path.*, 1914, lvii, 201.
20. Tschaschin, S., *Folia Haematol.*, 1913–14, xvii, 317.
21. Kiyono, K., Die vitale Karminspeicherung, Jena, 1914.
22. Ignatovski, *Izviest Imp. Voyenno-Med. Akad., S. Peterb.*, 1908, xviii, 231.
23. Ignatovski, A., *Virchows Arch. f. path. Anat.*, 1909, cxcviii, 248.
24. Anitschkow, *Deutsch. med. Wchnschr.*, 1913, xxxix, 741.
25. Rothschild, M. A., *Beitr. z. path. Anat. u. z. allg. Path.*, 1914, lx, 39.
26. Chalatow, *Beitr. z. path. Anat. u. z. allg. Path.*, 1914, lvii, 85.
27. Ophüls, W., *Proc. Soc. Exper. Biol. and Med.*, 1909–11, vii, 75.
28. Chalatow, *Virchows Arch. f. path. Anat.*, 1914, ccxvii, 351.
29. Zinserling, W. D., *Centralbl. f. allg. Path. u. path. Anat.*, 1913, xxiv, 627.

FIG. 1.

FIG. 2.

(Bailey: Atheroma Produced by Cholesterol Feeding.

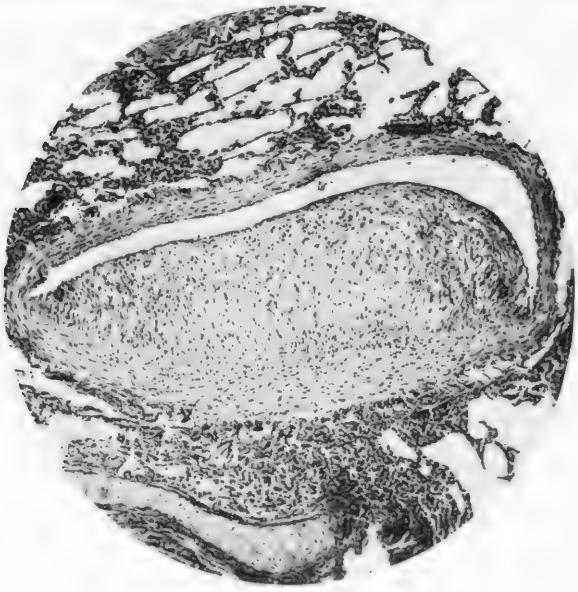

FIG. 3.

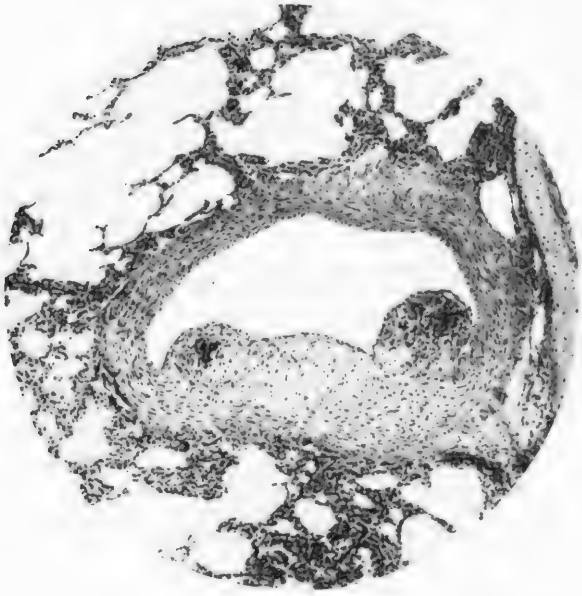

FIG. 4.

(Bailey: Atheroma Produced by Cholesterol Feeding.)

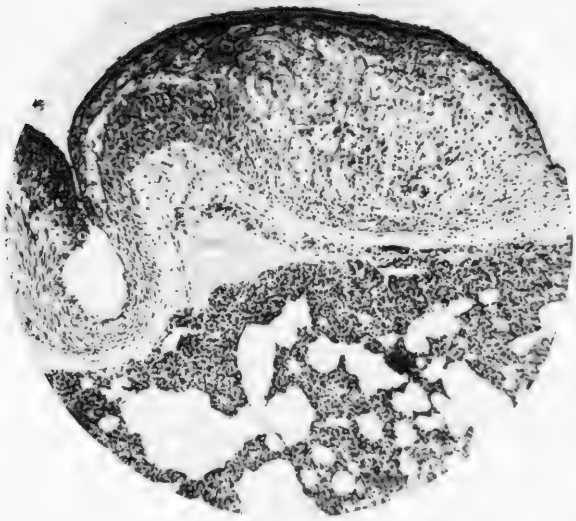

FIG. 5.

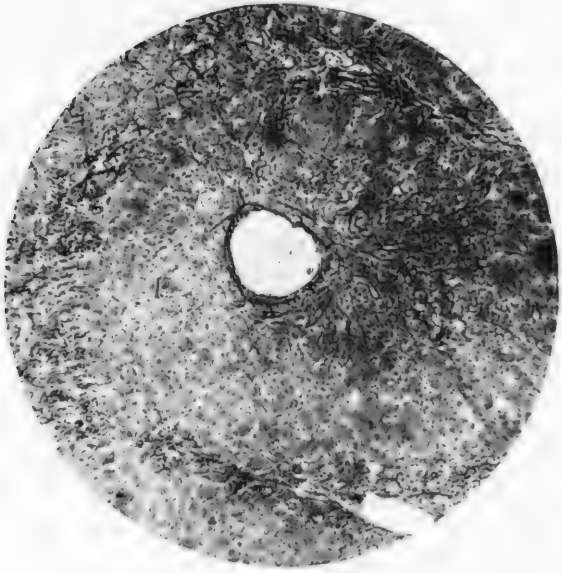

FIG. 6.

(Bailey: Atheroma Produced by Cholesterol Feeding.)

FIG. 7.

FIG. 8.

(Bailey: Atheroma Produced by Cholesterol Feeding.)

FIG. 9.

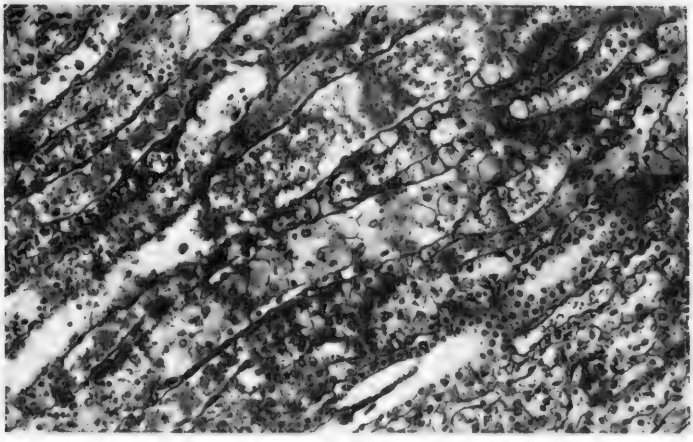

FIG. 10.

(Bailey: Atheroma Produced by Cholesterol Feeding.)

EXPLANATION OF PLATES.

PLATE 15.

FIG. 1. Rabbit 9. Thoracic portion of aorta. Shows large elevated plaques in the upper part; smaller lesions below.

FIG. 2. Rabbit 9. Aorta. Shows the edge of an area of atheroma with fibrosis on the surface and degeneration with cholesterol crystal spaces below. Slight invasion of media.

PLATE 16.

FIG. 3. Rabbit 11. Branch of pulmonary artery. Shows a pronounced nodular intimal thickening made up of the large cells which contain the anisotropic fat.

FIG. 4. Rabbit 9. Branch of pulmonary artery. Shows the large cells and calcification in an intimal lesion.

PLATE 17.

FIG. 5. Rabbit 9. Branch of pulmonary artery. Shows lesions composed of large cells, with fibrosis on surface, cholesterol crystal spaces, and some calcification below. Mainly intimal, but invading media.

FIG. 6. Rabbit 5. Liver. Shows a central vein with fine connective tissue fibrils extending outward into the lobule.

PLATE 18.

FIG. 7. Rabbit 9. Kidney. Shows the scars on the surface and the nodular lesions involving a large portion of the outer zone of the medulla and extending as fine lines into the inner zone.

FIG. 8. Rabbit 5. Kidney. Shows one of the nodular lesions in the outer zone of the medulla, which consist of large cells loaded with anisotropic fat.

PLATE 19.

FIG. 9. Rabbit 11. Kidney. One of the more pronounced lesions in the outer zone of the medulla. Many of the large cells are seen which contain the anisotropic fat; also areas of degeneration with numerous cholesterol crystal spaces. Surrounding the lesion there is moderate fibrosis. In the lesion a few collapsed tubules still persist.

FIG. 10. Rabbit 5. Kidney. Shows a capillary in the inner zone of the medulla containing many large cells. These cells are filled with anisotropic fat.

THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

XIV. Changes in the Blood Following Diversion of the Splenic Blood from the Liver. A Control Study of the Effects of Splenectomy.

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In the earlier publications from this laboratory (1) have been described the various phenomena which follow splenectomy in the dog. In the discussion of the most striking of these,—anemia, increased resistance of the red cells, decreased tendency to jaundice, and prolongation of the recovery from anemia—it was assumed that, inasmuch as the spleen was absent, these changes were probably due to the loss of some function peculiar to this organ. As to the exact nature of the function lost, no definite opinion has thus far been given. Three possibilities, however, readily suggest themselves: (1) the loss of some function of blood destruction or regeneration resident in the spleen itself; (2) the loss of an internal secretion acting on the distant hemopoietic tissues, such as the bone marrow; and (3) the alteration of the venous drainage of the spleen, which, in that it is an important source of the portal blood, may have an essential relation to some peculiar function of the liver.

It is obvious that the value of some of these hypotheses might be tested without removal of the spleen, by diverting its venous outflow to the liver. We have, therefore, repeated many of our experiments, but instead of removing the spleen, the splenic vein has either been ligated or transplanted into the vena cava, or an Eck fistula has been established. As far as we know, exactly similar experiments

have not previously been attempted, though two observations have a bearing on the problem. Contrary to our results, Nassau (2) found that the number, color, and resistance of the red cells remained unchanged after simple Eck fistula; but as he gives only one count before and one after the operation, his observations are not conclusive. The other report which concerns us is Pribram's (3) artificial constriction of the splenic vein with resultant passive congestion of the spleen. The slight anemia that this caused is analogous to some of the results we report below.

If the anemia following splenectomy is caused by the removal with the spleen of some necessary factor in blood formation or of a hormone essential to the hemopoietic tissues, this factor should not operate in the vein transplantation experiments and Eck fistula, unless it is also essential that such a substance have direct approach to the liver for its proper functioning or activation. If, on the other hand, the anemia is largely due to the interference with the supply of splenic blood to the liver, it should occur in all animals tested. These problems, and also that concerning the influence of the experimental procedures on increased resistance of the red cells, will be considered in this paper. Other problems such as the decreased tendency to jaundice and the prolonged repair of anemia in the splenectomized animal when hemolytic agents are given, will be presented in a later communication.

Methods.

In the ligation experiments it was found necessary, on account of the numerous branches and the anastomoses of the splenic vein with veins from the stomach, to tie all branches of the splenic vein shortly after they left the spleen. In the earlier operations one of the subdivisions of the artery was also ligated, to lessen the supposed danger of rupture. This, later, was abandoned when it was found to be unnecessary, as well as undesirable, on account of infarct production. Postmortem examination of animals subjected to this operation showed in each case that all branches had been tied, that the vessel beyond the ligation was much diminished in size or completely obliterated, and that there was usually little attempt at compensatory development of new veins from the adherent omentum. When such

new-formed veins were present it was found that they seldom emptied into the portal system, and therefore, as a rule, did not complicate the experiment. Eck fistula was performed in the usual manner, the proximal end of the portal vein being tied off above its new anastomosis with the vena cava just before its entrance into the liver. Transplantation of the splenic vein into the inferior vena cava presented considerable difficulty on account of the smallness of the vein and the necessity of stretching it a little to make it reach the vena cava. In only one experiment, however, was it found that the transplanted vein had been occluded by thrombus. The success of the operation was always determined by examination of the vessels at autopsy, and in some instances injection specimens were prepared and dissected to make doubly sure that no new anastomosis had formed.

The particular phenomena studied were: (1) the quantitative changes in the red blood cells, leukocytes, and hemoglobin; (2) the resistance of the red cells to hypotonic salt solution; (3) the general condition as indicated by the weight of the animals and condition of the urine; and (4) the gross and microscopical appearance of the spleen and other viscera at autopsy.

The dogs have been kept on the usual mixed diet of table scraps. This has been shown to maintain properly normal dogs as well as those convalescent from operations other than splenectomy, without the development of anemia (4).

RESULTS.

Our studies have been made on 15 dogs, divided as follows: ligation of splenic vein, 4; transplantation of splenic vein, 4; Eck fistula, 3; and, as controls, splenectomy, 3; transplantation of the inferior mesenteric vein, 1. In the case of the last dog, it was intended to transplant the splenic vein, and it was not until autopsy that we found that the inferior mesenteric vein had been used by mistake. As some of these dogs were used also for the study of the effect of hemolytic agents, all are not presented in this paper.

Changes in the Spleen.

In those dogs in which all branches of the splenic vein had been ligated and in which adequate new venous channels have failed to develop, the spleen shows considerable change. Before the operation is completed the spleen increases to almost double its size and takes on a dark purple color. If the animal is allowed to survive two or more months, the spleen at autopsy is found to be considerably smaller and much firmer than normal. The capsule is slightly thickened and puckered and the organ has a pale bluish color. On section the tissue cuts with increased resistance and a few small shrunken infarcts are occasionally found. The cut surface is less bloody than usual and shows an increase of fibrous tissue, with indistinct Malpighian corpuscles. Histologically, the tissue appears to be condensed with collapsed sinuses rather than to exhibit any marked fibrous tissue hyperplasia. Some hemosiderin pigment is present, the Malpighian corpuscles are small, and here and there are several areas of hyaline degeneration, which do not respond to the test for amyloid. Thromboses are not found, except in connection with the old postoperative infarcts. In the earlier animals, in which a branch of the artery was also ligated, a bulging hemorrhagic infarct appeared in the corresponding area. The abdominal lymph nodes and the liver are apparently unchanged. Speculation as to the adjustment of the splenic circulation in these cases must remain unsatisfied. The most probable explanation seems to be that the elastic spleen is able not only to accommodate the increasing pressure without rupture, but, with the aid of the minute new-formed capillaries in the adherent omentum, to maintain sufficient degree of nutrition to prevent necrosis. We have seen, however, that the exchange is not sufficient to prevent atrophy.

At the time of the operations for Eck fistula and the splenic vein-vena cava anastomosis, there is necessarily a temporary occlusion of the large veins. The spleen and intestine become very dark and turgid, but when the clamps are removed, these organs quickly return to normal color. At autopsy on animals so treated, except for numerous adhesions and frequently perisplenitis, no noteworthy changes are found.

Changes in the Blood.

An example of the changes in the blood which occur after ligation of the splenic vein is given in Table I.

It may be seen that this animal developed a moderate degree of anemia lasting several weeks and similar to but less severe than that following splenectomy. The anemia is accompanied by a temporary slight increase in resistance of the red cells to hypotonic salt solution.

TABLE I.

Blood Changes Following Ligation of Splenic Veins.

Dog 47.	Hemoglobin.	Red blood cells per cmm.	Hemolysis.		Leukocytes per cmm.	Polymorphonuclears.	Small lymphocytes.	Large transitionals.	Eosinophils.	Weight.
			Begins.	Complete.						
	per cent									*kilos*
Before	102	6,275,000	0.475	0.35	9,800	7,200	700	1,800	100	6.0
1 day after	98	6,110,000			19,000	15,200	950	2,850	0	5.7
4 days "	92	5,350,000	0.475	0.275	16,500					5.6
1 wk. "	90	5,520,000	0.425	0.275	16,800	13,600	1,300	1,600	300	5.4
2 " "	72	4,900,000	0.425	0.3	18,800	15,400	1,900	1,500	0	
3 " "	70	4,600,000	0.45	0.3	21,000	16,800	3,100	300	300	
4 " "	78	5,000,000			18,600					6.4
5 " "	74	4,380,000			15,000					
7 " "	80	5,040,000	0.475	0.275	12,800	10,000	1,700	1,100	0	7.3
10 " "	82	5,050,000	0.45	0.3	12,400					
12 " "	95	5,830,000	0.475	0.325	14,800	9,500	3,400	1,500	400	7.5
13 " "	92	6,410,000	0.475	0.35	14,900	9,200	3,600	1,200	900	
14 " "	95	6,150,000			15,600	12,200	2,500	600	100	7.4

As in splenectomy, there is an immediate leukocytosis, due to polymorphonuclear and transitional forms, but prolonged by a more persistent lymphocytosis and eosinophilia. A very slight drop in weight occurs after operation, but later there is a distinct increase above the original weight. It has been shown elsewhere (4) by the study of control operations such as nephrectomy, that the changes here described are not merely postoperative. Other dogs with ligated veins gave similar results; such as Dog 51, with a maximum drop of 14 per cent

of hemoglobin and 1,200,000 red cells per cmm.; Dog 74, hemoglobin loss 24 per cent, red blood cell loss 1,800,000 per cmm.

The effect of diverting the splenic venous blood from the liver by transplanting the splenic vein into the vena cava is shown in Table II.

Much the same changes as after ligation are found here. In another dog (No. 71) similarly treated, the increased resistance was much more marked: before operation, hemolysis began at 0.50 and was complete at 0.35; soon after operation, it began at 0.425 and was complete at 0.25. Anemia was also present (a drop of 20 per cent in hemoglobin and of 1,800,000 red blood cells). A third animal (No. 16) lost 35 per cent hemoglobin and over 2,000,000 erythrocytes, and a fourth (No. 24) 22 per cent hemoglobin and over 1,000,000 erythrocytes.

The changes in an Eck fistula experiment are seen in Table III.

In another Eck fistula dog (No. 68), the increased resistance of the red cells was more marked (before operation, hemolysis began at 0.45 and was complete at 0.3; soon after operation it began at 0.425 and was complete at 0.225) and the animal lost 33 per cent hemoglobin and 2,000,000 red blood cells, in four weeks' time after operation. In a third (No. 5) with an even greater anemia, the blood picture was complicated by the occurrence of infection, so the figures are not given.

That interference with inflow to the portal circulation, from organs other than the spleen is responsible for changes in the blood is shown in another experiment (Table IV) in which by accident an anastomosis of the inferior mesenteric vein was done.

This dog developed an anemia of mild grade and recovered from it sooner than did the other dogs of this series. Although little weight can be placed on a single experiment such as this, the observation tends to support the theory that the supply of portal blood to the liver is an important factor in our problem.

The urine remained without bile in all the animals used.

There was an occasional postoperative loss of weight (especially in some of the Eck fistula dogs), but this was by no means constant, and bore no apparent relation to the anemia. This was frequently followed by an increase to more than the original weight, just as is frequently found after splenectomy.

TABLE II.

Blood Changes Following Transplantation of the Splenic Vein.

Dog 4.	Hemoglobin.	Red blood cells per cmm.	Hemolysis.		Leukocytes per cmm.	Polymorphonuclears.	Small lymphocytes.	Large transitionals.	Eosinophils.	Weight.
			Begins.	Complete.						
	per cent									*kilos*
Before	97	6,960,000	0.45	0.35	16,100	8,400	4,700	1,600	1,400	9.4
1 day after	104	7,040,000	0.425	0.3	34,000	29,600	3,100	1,300	0	
1 wk. "	75	5,550,000	0.45	0.3	19,300	12,400	3,700	1,900	1,300	9.7
2 " "	68	5,020,000	0.475	0.275	16,900	11,800	2,700	2,200	200	9.4
3 " "	70	4,530,000								9.6
4 " "	80	4,510,000	0.475	0.3	14,700	9,600	2,800	1,500	800	10.4
5 " "	74	5,440,000	0.475	0.325	14,000	9,800	2,800	420	1,080	10.5
6 " "	78	5,630,000	0.45	0.3	11,200	6,300	4,000	560	360	
7 " "	82	5,770,000	0.45	0.325	11,100	7,400	2,900	200	600	12.9
8 " "	85	6,060,000	0.45	0.325						
9 " "	96	6,500,000								13.2

TABLE III.

Blood Changes Following Eck Fistula.

Dog 31.	Hemoglobin.	Red blood cells per cmm.	Hemolysis.		Leukocytes per cmm.	Polymorphonuclears.	Small lymphocytes.	Large transitionals.	Eosinophils.	Weight.
			Begins.	Complete.						
	per cent									*kilos*
Before	99	6,500,000	0.45	0.325	13,200	9,800	2,700	500	200	16.1
1 day after	88	6,040,000	0.475	0.325	36,000	32,400	2,800	800	0	
3 days "	84	6,300,000	0.45	0.325	32,400	27,500	2,900	1,600	400	14.2
5 " "	72	5,400,000	0.425	0.3	24,000	17,300	3,800	2,400	500	14.2
1 wk. "	74	5,500,000	0.425	0.3	18,800	13,700	3,400	800	900	13.6
2 " "	68	5,040,000	0.425	0.275	19,600	15,500	3,900	600	600	13.5
3 " "	73	5,110,000	0.425	0.275						
4 " "	67	4,460,000	0.425	0.3	21,000	15,500	4,400	600	500	14.6
5 " "	72	4,880,000	0.475	0.325	18,400	12,900	3,700	600	1,200	14.2
6 " "	84	5,600,000	0.475	0.325	16,000	10,500	3,800	400	1,300	14.9
7 " "	90	6,200,000	0.45	0.3	15,200	10,300	2,800	600	1,500	15.3
8 " "	98	6,520,000	0.45	0.3	15,800	11,400	3,500	300	600	15.4

TABLE IV.

Blood Changes Following Transplantation of Inferior Mesenteric Vein.

Dog 55.	Hemoglobin.	Red blood cells per cmm.	Hemolysis.		Weight.
			Begins.	Complete.	
	per cent				*kilos*
Before	100	5,940,000	0.45	0.3	14.7
2 days after	76	5,240,000	0.4	0.25	
3 " "	75	5,100,000	0.4	0.25	13.9
10 " "	76	4,880,000	0.45	0.275	
2 wks. "	85	5,400,000	0.425	0.25	
3 " "	84	5,890,000	0.425	0.25	
4 " "	78	5,600,000			14.7
5 " "	88	5,720,000			
6 " "	92	5,800,000	0.425	0.275	15.3

SUMMARY.

1. In dogs whose splenic veins have been ligated or transplanted into the inferior vena cava, or in which an Eck fistula has been made, an anemia occurs which resembles that following splenectomy and shows the same general variation in degree and duration.

2. The resistance of the red cells to hypotonic salt solution is quickly increased, sometimes coincident with and sometimes preceding the anemia. As a rule, it gradually returns to normal in about the same length of time as it takes the anemia to disappear, but may remain increased for longer periods.

3. There is an initial leukocytosis, involving at first the polymorphonuclear leucocytes and transitional cells. As the total leukocytosis diminishes there is both a relative and actual increase of small lymphocytes and usually of eosinophils. This may either be temporary or last during the rest of the period of observation and differs from the ordinary postoperative leukocytosis.

4. Ligation of the splenic vein is followed by considerable atrophy of the spleen, but not by necrosis or thrombosis. There is rarely adequate new vein formation. The other operations cause little or no change in the spleen.

5. Whether the disturbances as described are due to the loss of a certain volume of blood to the liver, or, as has been previously

suggested, to the loss of a splenic hormone, it is impossible to say. If the former is true, the method of production of the anemia still remains unexplained. It is evident, furthermore, that the latter theory has also no value unless it is assumed that this hormone must be activated by passage through the liver.

BIBLIOGRAPHY.

1. Pearce, R. M., Austin, J. H., and Krumbhaar, E. B., The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. I. Reactions to Hemolytic Serum at Various Intervals after Splenectomy, *Jour. Exper. Med.*, 1912, xvi, 363. Pearce, R. M., Austin, J. H., and Eisenbrey, A. B., II. The Relation of Hemoglobinemia to Hemoglobinuria and Jaundice in Normal and Splenectomized Animals, *Jour. Exper. Med.*, 1912, xvi, 375. Pearce, R. M., Austin, J. H., and Musser, J. H., Jr., III. The Changes in the Blood Following Splenectomy and Their Relation to the Production of Hemolytic Jaundice, *Jour. Exper. Med.*, 1912, xvi, 758. Pearce, R. M., and Austin, J. H., V. Changes in the Endothelial Cells of the Lymph Nodes and Liver in Splenectomized Animals Receiving Hemolytic Serum, *Jour. Exper. Med.*, 1912, xvi, 780. Musser, J. H., Jr., and Krumbhaar, E. B., VI. The Blood Picture at Various Periods after Splenectomy, *Jour. Exper. Med.*, 1913, xviii, 487. Pearce, R. M., and Peet, M. M., VII. The Effect of Hemolytic Serum in Splenectomized Dogs, *Jour. Exper. Med.*, 1913, xviii, 494. Krumbhaar, E. B., Musser, J. H., Jr., and Pearce, R. M., VIII. Regeneration of the Blood of Splenectomized Dogs after the Administration of Hemolytic Agents, *Jour. Exper. Med.*, 1913, xviii, 665. Pearce, R. M., and Pepper, O. H. P., IX. The Changes in the Bone Marrow after Splenectomy, *Jour. Exper. Med.*, 1914, xx, 19. Krumbhaar, E. B., and Musser, J. H., Jr., X. Concerning the Supposed Regulatory Influence of the Spleen in the Formation and Destruction of Erythrocytes, *Jour. Exper. Med.*, 1914, xx, 108. Austin, J. H., and Pearce, R. M., XI. The Influence of the Spleen on Iron Metabolism, *Jour. Exper. Med.*, 1914, xx, 122.
2. Nassau, E., Das Blutbild beim Hunde mit Eckscher Fistel, *Arch. f. exper. Path. u. Pharmakol.*, 1914, lxxv, 123.
3. Pribram, B., Hypersplenische Hämophthisen und Stauungsmilz, *Wien. klin. Wchnschr.*, 1913, xxvi, 1607.
4. Pearce, R. M., Austin, J. H., and Pepper, O. H. P., XV. The Influence of Diet upon the Anemia Following Splenectomy, *Jour. Exper. Med.*, 1916, xxii, 682.

THE RELATION OF THE SPLEEN TO BLOOD DESTRUCTION AND REGENERATION AND TO HEMOLYTIC JAUNDICE.

XV. The Resistance to Hemolytic Agents of Dogs in Which the Splenic Blood Has Been Diverted from the Liver.

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In a previous paper (1) has been discussed the effect upon the blood picture of three methods (transplantation of the splenic vein into the vena cava, Eck fistula, and ligation of the splenic veins) of diverting the splenic blood from the liver. In other papers (2) of this series have been described the decreased tendency to jaundice in splenectomized dogs receiving hemolytic serum and the slow repair of the accompanying anemia. In the present paper we shall present the results of a study of these two phenomena in animals whose splenic blood supply has been diverted from the liver, as above described.

Methods.

As hemolytic agents we have employed both toluylenediamin and hemolytic immune serum. The hemolytic serum was prepared by injecting at regular intervals the erythrocytes of the dog into rabbits. The serum so prepared was always injected intravenously into the dog under ether anesthesia. The other hemolytic agent, Merck's meta toluylenediamin, was given by stomach tube and in adequate doses never failed to cause anemia and jaundice. In each experiment the control animal received the same proportionate amount of drug or serum per kilo as did the test animals. Dogs of approximately the same size were selected and, on account of the occasional necessity of catheterization, female

dogs were used when possible. Splenectomized as well as normal animals were included in the series to further comparison with our previous results. Daily and later weekly examinations of urine and complete blood examinations (including resistance of erythrocytes to hypotonic salt solution) were made and records of weight were kept. Each animal was studied as to condition of urine and blood before the experiment began. Both cage urine and catheterized specimens (in doubtful cases) were examined, and the presence of bile pigment in the urine, as determined by Gmelin's and Rosenbach's tests, was taken as the surest evidence of jaundice. At the termination of the experiment the test animals were killed in order to determine the exact anatomical disturbance caused by the operation.

RESULTS.

Decreased Tendency to Jaundice.

In the animals with ligation of the splenic vein, it was occasionally found at autopsy that a small number of new blood vessels had forced their way through the splenic attachments, thus defeating the object of the experiment. In a previous paper we stated that new vein formation was inadequate to prevent atrophy of the spleen or the development of anemia. The mechanical vascular connection with the liver once being reestablished, however, these animals are apparently able to react with well marked jaundice as do normal dogs.

As may be seen in Table I, in all animals receiving adequate toluylenediamin, control as well as test dogs, some bile appeared in the urine. Frequently also well marked staining of the skin and scleræ persisted for many days. The bile was less in amount, however, and lasted for shorter periods in the test animals than in the normal controls. In fact, the animals with the vein transplant and Eck fistula showed even less tendency to jaundice than some of the splenectomized animals. Increased resistance of the red cells to hypotonic salt solution and the presence of anemia also contributed to diminish the jaundice; but it was shown that lessened tendency to jaundice might nevertheless be present in the absence of these features. For example, Dog 71, which had completely recovered from its anemia, showed the least tendency to jaundice, and Dog 79 which

TABLE I.

Jaundice after Administration of Toluylenediamin (0.34 Gm. per Kilo).

Time after administration.	Dog 71. Splenic vein transplant (2 mos.).	Dog 79. Splenectomy (1½ mos.).	Dog 51. Ligation (2 mos.).	Dog 75. Normal control.
	0 425 B. H.–0.325 C. H.* No anemia.	0.40 B.H.–0.275 C. H. No anemia.	0.45 B. H.–0.275 C. H. Slight anemia.	0.425 B. H.–0.275 C. H.
Before	Urine normal.	Urine normal.	Urine normal.	Urine normal.
1 day after	Bile trace.	Bile slight.	Bile moderate.	Bile marked.
3 days "	" heavy trace.	" "	" "	" "
5 " "	" trace.	" moderate.	" "	" "
7 " "	" absent.	" "	" "	" "
9 " "	" "	" "	" "	" moderate.
11 " "	" "	" trace.	" faint trace.	" trace.
13 " "	" "	" "	" absent.	" "
15 " "	" "	" "	" "	" "
17 " "	" "	" absent.	" "	" absent.

*These figures in this and other tables indicate strength of salt solution at which hemolysis occurs; B. H, indicates beginning hemolysis, C. H. complete hemolysis. Increasing amounts of bile in the urine are expressed in the following terms: faint trace, trace, heavy trace, slight, moderate, marked.

had the most resistant red cells, showed the same tendency to jaundice as did the control.

In another experiment (Table II), in which a smaller single dose was administered, similar results were obtained. It was in this

TABLE II.

Jaundice after Administration of Toluylenediamin (0.13 Gm. per Kilo).

Time after administration.	Dog 68. Eck fistula (1 mo.).	Dog 55. Mesenteric vein transplant (1½ mos.).	Dog 77. Normal control.
	0.4 B.H.–0.275 C. H. Anemia present.	0.425 B. H.–0.275 C. H. No anemia.	0.425 B. H.–0 35 C. H.
Before	Urine normal.	Urine normal.	Urine normal.
1 day after	Bile absent.	Bile trace.	Bile trace.
2 days "	" faint trace.	" "	" marked.
3 " "	" " "	" moderate.	" moderate.
5 " "	" absent.	" doubtful.	" "
7 " "	" "	" absent.	" trace.
9 " "	" "	" "	" absent.

experiment that an animal that was supposed to have had the splenic vein transplanted was found at autopsy to have a branch of the inferior mesenteric vein transplanted by mistake, thus unwittingly causing an excellent double control for the Eck fistula test (No. 68). The increased resistance to jaundice in this dog (No. 55) as compared to the normal (No. 77) is still further evidence of the importance of the mechanical factor of blood supply to the liver.

In a third experiment, in which three smaller repeated doses were given to animals with (1) splenic vein transplant; (2) splenic vein ligation; and (3) a normal control, these results were confirmed. The vein transplant dog, although his red cells at the time were the least resistant of the three, failed to get jaundice; in the other two it did occur. The jaundice in the ligation experiment, however, lasted three days and that of the control nine days,

In the experiments with hemolytic immune serum the same results were obtained. In a preliminary experiment, in which large doses of serum were given, an Eck fistula dog alone survived and developed jaundice; one with the vein transplant and the control died in a few hours; the control, however, had already developed hemoglobinuria, whereas the test dog failed to develop either hemoglobinuria or jaundice.

With a weaker serum (Table III) the greater resistance of the vein transplant animal is shown. A result contrary to our former experiences was obtained in this experiment, in that No. 2 (splenectomized) developed as severe a jaundice as did the control. A fourth animal (not given in the table) whose splenic vein had been ligated succumbed to an equivalent dose before jaundice developed. At autopsy the finding of new-formed veins was considered to render the experiment equivalent to one on a normal dog.

In another experiment (Table IV) the same results were obtained with a serum of less hemolytic power.

As the transplanted vein was found at autopsy to have been occluded by a comparatively recent thrombus (probably antedating the administration of serum), this experiment must be regarded as including a double test of obstruction to the venous outflow from the spleen. The relatively greater resistance to jaundice of No. 16 as compared with No. 51 may be partly explained by the greater resist-

TABLE III.

Jaundice after Administration of Hemolytic Serum (Serum No. 3, 0.4 Cc. per Kilo).

Time after administration.	Dog 4. Vein switch (2 mos.).	Dog 2. Splenectomy (2½ mos.).	Dog 25. Normal control.
	0.45 B. H.–0.325 C. H. Slight anemia.	0.425 B. H.–0.25 C. H. Slight anemia.	0.475 B. H.–0.35 C. H.
Before	Urine normal.	Urine normal.	Urine normal.
1 day after	No bile.	Bile moderate.	Bile trace.
2 days "	" "	" "	" moderate.
4 " "	" "	" "	" absent.
6 " "	" "	" absent.	

TABLE IV.

Jaundice after Administration of Hemolytic Serum (Serum No. 4, 0.2 Cc. per Kilo).

Time after administration.	Dog 16. Vein transplant (occluded vessels) (1 mo.).	Dog 1. Splenectomy (3 mos.).	Dog 51. Ligation (6 mos.).	Dog 27. Normal control.
	0.4 B. H.–0.25 C. H. Anemia present.	0.4 B. H.–0.25 C. H. Slight anemia.	0.45 B. H.–0.325 C. H. No anemia.	0.45 B. H.–0.35 C. H.
Before	Urine normal.	Urine normal.	Urine normal.	Urine normal.
1 day after	No bile.	No bile.	No bile.	Bile marked.
2 days "	" "	" "	Bile marked.	" moderate.
3 " "	" "	" "	" "	" trace.
5 " "	" "	" "	" "	" absent.
			(Bile still present on the 14th day.)	

ance of the red cells in the former animal, but the longer duration of jaundice in No. 51 than in the control must be noted as an exception to the general rule.

The results of these experiments, therefore, indicate that the mechanical factor of the method of the blood's approach to the liver is of importance in determining the degree of resistance to hemolytic agents. Our results in this regard are in accord with those obtained by Pepper and Austin (3) on the influence of the site of hemoglobin injections on the production of jaundice.

Duration and Repair of Anemia after Administration of Hemolytic Agents.

Interpretation of the severity and duration of the anemia caused in the various test animals is complicated by several factors. In the first place the original operation has been shown (1) to cause anemia. As the test animals were sometimes given the hemolytic agent while more or less anemic, we have had to analyze our results with constant reference to this factor. Moreover, we have found that in most cases the severity of the anemia is usually the converse of the resistance of the red cells. In some cases, however, animals with the most fragile cells developed the least anemia after administration of hemolytic agents. Changes in weight in the animals of these experiments seem to bear no definite significant relation to the anemia.

We have previously stated (4) that the anemia caused in splenectomized animals by hemolytic agents is, as shown by direct blood examination, of greater severity, runs a longer course, and has a longer period of repair than is the case in the normal dog. By greater severity was meant that the hemoglobin and red cell count reached lower figures than in the control. The actual blood destruction, however, (taken by estimating the drop from the condition immediately before administering hemolytic agents) was usually less in splenectomized than in normal dogs. Our experiments of this year show that the same statements hold true after transplantation of the splenic vein into the vena cava, Eck fistula, and ligation of the splenic veins. The "actual blood destruction" of the test animals has been constantly less than in the normal controls, and in a few instances even the degree of anemia as expressed by the hemoglobin and red cell count was less severe in the test animals. The "greater severity" of the anemia must therefore be considered as open to question, and the "actual blood destruction" in the test animals less than in the normal controls. This, therefore, removes an apparent contradiction in our former work; namely, that although the splenectomized animal showed less signs of blood destruction (jaundice), nevertheless its anemia was more severe than that of the normal control.

The duration of the anemia of the test animals has been longer than that of the normal controls and paralleled that of the splenectomy controls. The difference, however, of both test and splenectomy dogs from the normal controls has been less striking than in our previous work; and like the variable degree of anemia after splenectomy, must be referred to changes in unknown factors (possibly such as diet or season or differences in toxicity of the serum).

The slowness of repair after interference with the splenic function, as contrasted with that in the normal animal is shown in Table V. The red cells show the same temporary decreased resistance to hypotonic salt solution, as has been previously commented upon (3).

In the second experiment, the red cells of No. 68 (Eck fistula) dropped 1,000,000 less than the control, but took 2 months for recovery, as opposed to 19 days of the control. In a third experiment smaller doses of the third series failed materially to affect the blood picture.

A similar experiment with hemolytic immune serum is presented in Table VI.

In a fourth experiment a weaker serum failed to cause anemia in a splenectomized dog as well as in one with occluded splenic vein transplant; however, an animal with ligated splenic vein developed about the same amount of anemia as the control, but took much longer to recover.

Behavior of Leukocytes.

Both toluylenediamin and hemolytic immune serum cause a marked leukocytosis, reaching its height in one to three days and lasting two to four weeks. The first and greatest rise (actual and relative) is in the polymorphonuclears and transitional cells; this is later followed by a less marked and more persistent rise of small lymphocytes and eosinophils. There was no essential difference in the reaction of the test dogs and their controls, except that the latter were still affected by doses too small to affect the former. These changes resemble those described by us in the leukocytes following the various operative procedures on the spleen, and probably indicate a general rather than any specific interference with the leukocytic elements of the blood.

TABLE V.

Duration and Severity of Anemia after Administration of Toluylenediamin (0.34 Gm. per Kilo).

Time after administration.	Dog 71. Splenic vein transplant.			Dog 79. Splenectomy.		
	Hemoglobin.	Red blood cells.	Resistance.	Hemoglobin.	Red blood cells.	Resistance.
	per cent		*B.H. C.H.*	*per cent*		*B.H. C.H.*
Before	98	6,785,000	0.45 –0.325	74	5,720,000	0.4 –0.275
1 day after	102	6,940,000	0.5 –0.3	84	4,770,000	0.5 –0.275
3 days "	58	5,490,000	0.475–0.275	60	4,250,000	0.425–0.275
5 " "	68	5,340,000	0.475–0.325	43	3,380,000	0.45 –0.25
7 " "	72	4,820,000	0.475–0.325	52	3,820,000	0.45 –0.25
9 " "	83	5,070,000	0.5 –0.3	63	4,080,000	0.425–0.25
12 " "	96	5,890,000	0.5 –0.3	66	4,320,000	0.425–0.25
16 " "	98	6,210,000		69	4,530,000	
22 " "	96	6,350,000	0.5 –0.3	68	4,510,000	0.4 –0.275
29 " "				76	5,280,000	0.4 –0.25
35 " "			0.5 –0.3	88	5,590,000	0.4 –0.275
41 " "			0.475–0.325	88	5,480,000	0.4 –0.35
49 " "	98	6,800,000		92	5,680,000	0.4 –0.25

Time after administration.	Dog 51. Ligation.			Dog 75. Normal control.		
	Hemoglobin.	Red blood cells.	Resistance.	Hemoglobin.	Red blood cells.	Resistance.
	per cent		*B.H. C.H.*	*per cent*		*B.H. C.H.*
Before	80	4,920,000	0.45 –0.275	85	6,180,000	0.425–0.275
1 day after	90	4,800,000	0.5 –0.325	96	5,080,000	0.45 –0.35
3 days "				48	4,590,000	0.475–0.275
5 " "	60	3,820,000	0.375–0.25	38	2,980,000	0.45 –0.3
7 " "				45	3,630,000	0.475–0.3
9 " "	62	3,310,000	0.45 –0.3	55	3,930,000	0.45 –0.3
12 " "	65	3,980,000	0.45 –0.275	68	4,800,000	0.475–0.3
16 " "	72	4,320,000		80	5,200,000	0.475–0.3
22 " "	74	4,650,000		85	5,820,000	0.425–0.3
29 " "	76	4,950,000	0.475–0.35			
35 " "	84	5,330,000				
41 " "	78	5,340,000	0.425–0.3			
49 " "	87	5,550,000	0.45 –0.3			

TABLE VI.

Duration and Severity of Anemia after Administration of Hemolytic Serum (0.4 Cc. per Kilo).

Time after administration.	Dog 4. Vein transplant (2 mos.).		Dog 2. Splenectomy (3½ mos.).		Dog 25. Normal control.	
	Hemoglobin.	Red blood cells.	Hemoglobin.	Red blood cells.	Hemoglobin.	Red blood cells.
	per cent		*per cent*		*per cent*	
Before	97	6,960,000	85	4,950,000	103	6,400,000
1 day after	83	4,840,000	78	4,920,000	82	5,040,000
6 days "	65	3,940,000	55	3,670,000	58	3,950,000
10 " "	62	3,750,000	67	3,890,000	78	4,480,000
16 " "	70	4,390,000	67	4,090,000	80	4,880,000
19 " "	90	5,060,000			93	5,490,000
26 " "	96	6,010,000	78	5,260,000	106	6,060,000
31 " "	102	6,400,000	82	5,590,000		

SUMMARY.

1. Dogs whose splenic veins or portal vein (Eck fistula) have been transplanted into the inferior vena cava, or whose splenic veins have been ligated, show a lessened tendency to jaundice similar to that exhibited by splenectomized animals.

2. Although the previously existing anemia and the concomitant increased resistance of the red cells of these animals are undoubtedly factors in the greater resistance to hemolytic agents, the lessened tendency to jaundice is, in part at least, due to a mechanical factor dependent on the course of the blood supply to the liver.

3. The additional anemia caused in the test animals by hemolytic agents is usually less than in the controls, although the total fall from the original normal may be greater. This applies to the splenectomized as well as the other test animals and is a modification of our former statements in regard to the severity of the anemia in splenectomized dogs.

4. Although the destruction of blood in these animals is less than in the normal controls, the repair of the same takes considerably longer than in the controls. This confirms similar results previously obtained in splenectomized animals.

5. The white cells exhibit much the same changes as they do following the administration of hemolytic agents to splenectomized or normal animals. As these changes are not unlike those following uncomplicated splenectomy or the operations here discussed, they cannot be considered as characteristic of any of the above procedures, but perhaps as an accompaniment of any temporary increased blood destruction.

6. The reaction of test and control animals is substantially the same, whether the jaundice is caused by toluylenediamin or hemolytic immune serum.

BIBLIOGRAPHY.

1. Krumbhaar, E. B., Musser, J. H., Jr., and Peet, M. M., The Relation of the Spleen to Blood Destruction and Regeneration and to Hemolytic Jaundice. XIV. Changes in the Blood Following Diversion of the Splenic Blood from the Liver. A Control Study of the Effects of Splenectomy, *Jour. Exper. Med.*, 1916, xxiii, 87.

2. Pearce, R. M., Austin, J. H., and Krumbhaar, E. B., I. Reactions to Hemolytic Serum at Various Intervals after Splenectomy, *Jour. Exper. Med.*, 1912, xvi, 363. Pearce, R. M., Austin, J. H., and Eisenbrey, A. B., II. The Relation of Hemoglobinemia to Hemoglobinuria and Jaundice in Normal and Splenectomized Animals, *Jour. Exper. Med.*, 1912, xvi, 375. Pearce, R. M., Austin, J. H., and Musser, J. H., Jr., III. The Changes in the Blood Following Splenectomy and Their Relation to the Production of Hemolytic Jaundice, *Jour. Exper. Med.*, 1912, xvi, 758. Pearce, R. M., and Peet, M. M., VII. The Effect of Hemolytic Serum in Splenectomized Dogs, *Jour. Exper. Med.*, 1913, xviii, 494.

3. Austin, J. H., and Pepper, O. H. P., XII. The Importance in the Producduction of Hemolytic Jaundice of the Path of Hemoglobin to the Liver, *Jour. Exper. Med.*, 1915, xxii, 675.

4. Krumbhaar, E. B., Musser, J. H., Jr., and Pearce, R. M., VIII. Regeneration of the Blood of Splenectomized Dogs after the Administration of Hemolytic Agents, *Jour. Exper. Med.*, 1913, xviii, 665.

A QUANTITATIVE ANALYSIS OF THE INFLUENCE OF THE SIZE OF THE DEFECT ON WOUND HEALING IN THE SKIN OF THE GUINEA PIG.

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The investigations of Loeb, Addison, and Spain have shown that normal as well as regenerative growth of the epidermis of various species of animals differs markedly, that there is a distinct relationship between the structures of the various skins and their proliferative energy, and that the structure of the normal skin and its behavior during regeneration are apparently determined by the proliferative energy of the cells.[1]

We undertook to determine the influence of the size of the wound on the rate of wound healing in the different types of skin, and in this communication we deal with the results obtained in the guinea pig. In one set of animals a thin flap of skin measuring approximately 2 sq. mm. was removed from the ear of each animal; in another set flaps measuring 4 sq. mm. were removed. After 2, 5, 7, 9, 11, and 14 days pieces were cut out in each set for microscopic examination. Three to five pieces were examined at each period, in both the 2 sq. mm. and the 4 sq. mm. series. All the pieces were cut into several sections. The same measurements were made as in the previous investigations. The results are given in Tables I, II, III, and IV.[2]

[1] Loeb, L., and Addison, W. H. F., *Arch. f. Entwcklngsmechn. d. Organ.*, 1911, xxxii, 44. Addison, W. H. F., and Loeb, L., *Arch. f. Entwcklngsmechn. d. Organ.*, 1913, xxxvii, 635. Spain, K. C., *Jour. Exper. Med.*, 1915, xxi, 193.

[2] The microscopic measurements were carried out by Dr. Spain.

TABLE I.

Four Millimeter Series.

Day.	No. of piece.	Length of tongue.	Length of defect.	Length of tongue and defect.	No. of living cell rows and width of stratum germinativum.		
					Tip of tongue.	Insertion of tongue.	Old epithelium.
		mm.	*mm.*	*mm.*			
2		0.498		3.952	1–3	4–5	4
	1	0.454	3.0		(14.6μ)	(47.1–53.8μ)	(41.3μ)
		0.526		3.954	1–3	4–5	4
	2	0.619	2.813		(15.3μ)	(49.53–57.6μ)	(45.3μ)
		0.462		4.086	1–3	4–5	4
	3	0.511	3.113		(16.1μ)	(42.7–56.9μ)	(51.4μ)
$3\frac{1}{2}$		0.0823			1–3	5–7	5
	1	0.0965	2.0	3.788	(19.2μ)	(55.2–67.4μ)	(56.9μ)
					Middle of wound.		
5	1	Closed		2.137	7	7–11	7
					(76.3μ)	(69.2–117.3μ)	(64.7μ)
	2	"		2.167	6	7–11	7
					(73.9μ)	(88.4–119.3μ)	(68.7μ)
	3	"		2.772	6	7–11	7
					(69.2μ)	(84.0–126.9μ)	(65.3μ)
7	1	"		1.567	7	5–7	5
					(70.3μ)	(57.2–83.4μ)	(53.8μ)
	2	"		1.662	6	5–7	5
					(62.8μ)	(62.6–93.3μ)	(53.4μ)
	3	"		1.852	6	5–7	5
					(61.7μ)	(61.5–88.1μ)	(56.1μ)
9	1	"		1.514	5	5–7	5
					(58.0μ)	(59.2–79.6μ)	(46.2)
	2	"		1.715	5	5–7	5
					(55.6μ)	(48.5–65.7μ)	(48.4μ)
	3	"		1.562	4	5–7	4
					(41.1μ)	(52.6–64.4μ)	(39.6μ)
11	1	"		1.372	3	5–7	4
					(32.5μ)	(42.3–54.5μ)	(37.3μ)
	2	"		1.309	3	5–7	4
					(29.6μ)	(49.9–67.6μ)	(45.7μ)
	3	"		1.330	3	5–7	4
					(33.8μ)	(50.9–61.3μ)	(38.4μ)
14	1	"		1.228	3	5–7	4
					(26.9μ)	(53.4–60.7μ)	(39.9μ)
	2	"		1.086	3	5–7	4
					(30.7μ)	(48.6–57.4μ)	(34.6μ)
	3	"		1.079	3	5–7	4
					(28.5μ)	(51.9–55.3μ)	(43.0μ)

TABLE II.

Four Millimeter Series.

Day.	No. of piece.	Size of cell.	Size of nucleus.	No. of mitoses in one area 1 mm. long x 50μ wide.	
				New epithelium.	Old epithelium.
2	1 2 3	μ 15.3 x 9.6	μ 11.6 x 7.6	0 1 } $\frac{1}{3}$ 0	29 28 } $29\frac{2}{3}$ 32
$3\frac{1}{2}$	1 2 3	16.3 x 9.7	11.8 x 7.5	38	30
5	1 2 3	12.1 x 9.7	9.1 x 6.5	4 7 } $6\frac{1}{3}$ 8	15 12 } $13\frac{2}{3}$ 14
7	1 2 3	12.6 x 9.8	9.3 x 6.9	4 4 } 5 7	15 16 } $15\frac{1}{3}$ 15
9	1 2 3	11.9 x 9.6	9.2 x 6.9	4 5 } $4\frac{2}{3}$ 5	14 13 } 14 15
11	1 2 3	11.8 x 9.7	8.7 x 6.4	4 3 } $3\frac{1}{3}$ 3	13 12 } 12 11
14	1 2 3	11.2 x 9.7	8.9 x 6.1	4 3 } $3\frac{1}{3}$ 3	13 12 } $12\frac{2}{3}$ 13

Changes in the Size of the Wound and Tongue.

The various headings under which the data are arranged in these tables are the same as in the tables in our preceding communications.[3] Although the figures of only three pieces are given in the tables, in the majority of cases additional pieces were examined, and on the whole the results were concordant. In the third column of Tables I and III we find the figures for the length of the tongue in the various periods. Two days after making the wound a tongue

[3] Addison, W. H. F., and Loeb, L., *Arch. f. Entwcklngsmechn. d. Organ.*, 1913, xxxvii, 635. Spain, K. C., *Jour. Exper. Med.*, 1915, xxi, 193.

TABLE III.

Two Millimeter Series.

Day	No. of piece.	Length of tongue.	Length of defect.	Length of tongue and defect.	No. of rows of living cells and width of stratum germinativum.		
					Tip of tongue.	Insertion of tongue.	Old epithelium.
		mm.	*mm.*	*mm.*			
2		0.30		2.03	1–2	3–4	3–4
	1	0.23	1.504		(13.4μ)	(30.6–43.8μ)	(34.7μ)
		0.38		1.956	1–2	3–4	3–4
	2	0.15	1.426		(12.6μ)	(28.7–45.6μ)	(37.8μ)
		0.25		2.23	1–2	3–4	3–4
	3	0.28	1.68		(11.5μ)	(38.4–41.9μ)	(33.6μ)
		0.265					
5		0.538		2.15	3–4	7–11	7–9
	1	0.682	0.83		(38.9μ)	(91.3–115.3μ)	(69.2μ)
		0.513		2.28	3–4	7–11	7–9
	2	0.787	0.984		(46.9μ)	(90.9–109.6μ)	(66.3μ)
		0.692		1.98	3–4	7–11	7–9
	3	0.511	0.782		(42.3μ)	(88.8–111.6μ)	(71.6μ)
		0.6250					
7	1	1.076	0.007	1.852	5–6	7–11	6–7
		0.769			(63.3μ)	(78.0–123.07μ)	(68.5μ)
		0.922			Middle of wound.		
	2	Closed		1.662	5–6	7–11	5–6
					(64.2μ)	(78.6–84.7μ)	(58.8μ)
	3	"		1.568	5–6	7–11	5–6
					(62.7μ)	(72.6–89.6μ)	(61.5μ)
9	1	"		1.461	3–4	7–9	4–5
					(46.1μ)	(69.3–78.4μ)	(48.4μ)
	2	"		1.388	3–4	7–9	4–5
					(52.3μ)	(72.3–84.2μ)	(49.9μ)
	3	"		1.569	3–4	7–9	4–5
					(48.2μ)	(60.8–80.7μ)	(52.8μ)
11	1	"		1.204	3–4	5–7	4–5
					(48.2μ)	(50.8–69.2μ)	(44.2μ)
	2	"		1.406	3–4	5–7	4–5
					(41.5μ)	(50.2–64.7μ)	(41.2μ)
	3	"		1.284	3–5	5–6	4–5
					(53.8)μ	(49.7–67.2μ)	(43.1μ)
14	1	"		1.208	3–4	5–7	3–4
					(37.9μ)	(46.1–57.6μ)	(36.4μ)
	2	"		1.197	3–4	5–7	3–4
					(38.4μ)	(42.3–61.5μ)	(44.2μ)
	3	"		1.228	3–4	5–7	3–4
					(42.3μ)	(51.9–63.8μ)	(35.3μ)

TABLE IV.

Two Millimeter Series.

Day.	No. of pieces.	Size of cell.	Size of nucleus.	No. of mitoses in one area 1 mm. long x 50 μ wide.	
				New epithelium.	Old epithelium.
2	1 2 3	μ 13.4 x 8.9	μ 9.2 x 7.3	1 2 } 1⅓ 1	44 45 } 44 43
5	1 2 3	14.6 x 9.9	10.3 x 7.9	28 26 } 26 24	26 23 } 24 23
7	1 2 3	Open 14.6 x 9.7 Closed 12.1 x 8.6	9.8 x 7.6 9.5 x 7.5	Open 72 Closed 42	26 20
9	1 2 3	13.4 x 8.8	9.5 x 7.2	27 22 } 22 17	20 16 } 17⅔ 17
11	1 2 3	11.7 x 7.5	9.1 x 6.6	15 14 } 15⅓ 17	14 15 } 13⅔ 12
14	1 2 3	11.2 x 7.3	9.3 x 6.7	11 14 } 12⅔ 12	13 14 } 14⅓ 16

of regenerating epidermis measures 0.265 mm. in the 2 mm. series, and almost twice as much in the 4 mm. series. After three and one-half days the tongue has increased considerably and it is closed after five days in the 4 mm. series. In the 2 mm. series the length of the tongue has enlarged considerably at this period. It is more than double as long as two days after the operation, but the wound is not yet closed. In two pieces the wound was closed after seven days, in one it was still open in the 2 mm. series. After nine days the wound is closed in all the series. The figures in the 3 mm. series, which Addison and Loeb communicated previously,[4] stand generally

[4] Addison, W. H. F., and Loeb, L., *Arch. f. Entwcklngsmechn. d. Organ.*, 1913, xxxvii, 635.

between the figures in the 2 and 4 mm. series. Slight variations which occur are probably due to the fact that in the previous work the exact size of the wound had as yet not been considered as of very great importance and there may therefore have been slight variations in the size of the original wound in the 3 mm. series. We may conclude from these figures that the larger the wound the more rapidly the tongue enlarges and the earlier the closure of the wound takes place, within the variations in size of the wound chosen in our experiments. This difference in the energy of outgrowth of the epithelial tissue is already noticeable within the first two days. Again, from the second to the fifth day the increase in size of the tongue over the size two days after the operation is absolutely greater in the 4 mm. than in the 2 mm. series.

A study of the changes in the size of the defects agrees with the changes in the size of the tongues (Column 4, Tables I and III). Two days after the operation the defect has decreased 1.025 mm. in the 4 mm. series and a little less than half as much (0.461 mm.) in the 2 mm. series. From two days to three and one-half days a further decrease of 0.81 mm. in the size of the defect has taken place in the 4 mm. series. The rate of the decrease is approximately the same as in the preceding period in the 4 mm. series. Sometimes between three and one-half and five days the defect disappears in the 4 mm. series. In the 2 mm. series it decreases 0.639 mm. from the second to the fifth day. Again the defect decreases from the second to the fifth day more in the 4 mm. than in the 2 mm. series. We find, therefore, that the decrease in the size of the defect is in the 4 mm. series very much more rapid than in the 2 mm. series. The figures for the 3 mm. wounds stand between those for 2 and 4 mm.

Contraction of Wound.—In the fifth column of Tables I and III we find the figures for the length of tongues plus defect or of the length of the former defect, after it has been covered by the tongues. The figures in this column after the closure of the wound and the difference between the decrease in the defect and the increase in the tongues in the different periods give an indication of the contraction which takes place in the area of the wound simultaneously with the outgrowth of the tongues.

If we consider first the 4 mm. series, we find no noticeable con-

traction before the end of the second day; between the second and third and a half day there is possibly a very slight contraction. There is a noticeable contraction beginning before the end of the fifth day. A decided contraction, therefore, takes place at the time of the closure of the wound and shortly preceding this period. Between the fifth and seventh day a further decided contraction takes place. Between the seventh and fourteenth day the contraction continues, but at a generally diminishing rate.

In the 2 mm. series the contraction begins much later than in the 4 mm. series, and again at the period of closure and the time preceding it; *viz.*, between the fifth and seventh days. During this period the contraction is quite noticeable. From the seventh to the fourteenth day the contraction continues here with gradually decreasing intensity. In the 4 mm., as well as in the 2 mm. series, the contraction is therefore most marked in the beginning and gradually decreases. At the time of closure the contraction amounts to about 0.4 to 0.5 mm. in both series. Between the ninth and eleventh days the contraction is in both cases somewhat greater than 0.2 mm. Fourteen days after the operation the area of the healed wound has shrunken in both series to about the same area. Therefore, the contraction that takes place in the 4 mm. series is absolutely much greater than that in the 2 mm. series. The amount of contraction in the two series differs, therefore, in the period preceding the seventh day. Between the seventh and fourteenth days the contraction is absolutely approximately the same in both series. The contraction is therefore greater in larger wounds. The approximate contraction in the two series is as follows (Table V).

TABLE V.

Days.	4 mm. series. *mm.*	2 mm. series. *mm.*
0 to 2		
2 to 3½	0.05	
3½ to 5	0.4	
5 to 7	0.68	0.5
7 to 9	0.09	0.22
9 to 11	0.22	0.23
11 to 14	0.14	0.04
Total	1.58	0.99

These figures show that both outgrowing of the tongues and contraction are concerned in the closing of the defect and that both are greater in the larger wound.

Mitoses.—The number of mitoses in the 4 mm. series is given in Table II, and in the 2 mm. series in Table IV. We distinguish between the mitoses in the tongue (here the number of mitoses in one of the tongues over an area 50 μ wide is counted) and in the old epithelium (the number of mitoses in the old epithelium is determined over an area extending 1 mm. from the insertion of the tongue into the old epithelium and 50 μ wide). After the closure of the wound the mitoses in half the epithelium covering the defect are counted.

At two days we find in the 4 mm. series in the tongue $\frac{1}{3}$ of a mitosis in one unit area, in the old epithelium 30 mitoses in a unit area; in the 2 mm. series there are $1\frac{1}{3}$ mitoses in one unit area in the tongue, and 44 mitoses in the old epithelium. As we stated in an earlier communication, at first there are almost no mitoses in the tongue and many mitoses in the old epithelium. In the animals with the smaller wounds there are more mitoses in the old epithelium, although the tongues are greater in the animals with the larger wounds. We may therefore conclude that the tongue formation is not directly dependent on the mitoses but on the migration of the cells. We notice furthermore that also in the tongue the mitoses are more frequent in the smaller wounds. Within a very short time the mitoses increase rapidly in the tongue, and at three and one-half days the mitoses are more frequent in the tongue than in the old epithelium in the 4 mm. series. In the old epithelium the number remains at three and one-half days about the same as at two days.

At five days the wound is closed in the 4 mm. series. We find therefore a sudden decline in the number of mitoses in both former tongue and old epithelium, but the decrease is greater in the tongue, where the pressure of cell layers which meet from opposite directions upon each other is greatest. In the 2 mm. series where the wounds are not yet closed the mitoses in the tongues increase markedly, just as the mitoses did in the tongues in the 4 mm. series at three and one-half days. In the old epithelium, however, no further increase, but a certain decrease takes place, which latter is, however, probably

not very significant and may perhaps be due to a relative diminution in the pull from the tongue.

At seven days the number of mitoses in the former tongue and old epithelium is similar to the number at five days in the 4 mm. series. In the 2 mm. series we must distinguish between those pieces in which a closure of the wound has not yet taken place, and those in which the wound has just closed. In the former the number of mitoses in the tongue increases very much (72), while in the old epithelium the number of mitoses has hardly undergone a change. In the latter directly after the closure a rapid decline in the number of mitoses in the tongues has set in; in these pieces the number of mitoses is about 50 per cent less than in the pieces in which the wound is not yet closed, while in the old epithelium only a relatively much smaller diminution has taken place after the closure (20 mitoses). In a similar way we found in the 3 mm. series a marked rise in the number of mitoses in the tongue and no increase, or rather a slight decline, in the old epithelium just before the time of closure of the wound. In the 4 mm. series the fall in the number of mitoses in the old epithelium directly after the closure of the wound is greater than in the 3 and 2 mm. series. In the latter series in the old epithelium a slight fall had already taken place before the closure of the wound.

At nine days the number of mitoses in the former tongue and old epithelium is about the same as at seven days; in the 4 mm. series there is at the most a very slight decrease. In the 2 mm. series there is a marked decrease in the number of mitoses in the old tongue and a slight decrease in the old epithelium. At both places, however, the number of mitoses is absolutely greater than after the closure of the wound in the 4 mm. series. In all three series the decrease in the number of mitoses after the closure of the wound is greatest in the tongue, while the number of mitoses in the old epithelium is subject to much less marked variations. We see that absolutely the number of mitoses remains higher in the 2 mm. than in the 4 mm. series. Thus the figures for the mitoses at nine days in the former tongues are $4\frac{2}{3}$ and 22 mitoses, and for the old epithelium 14 and $17\frac{2}{3}$ mitoses in the 4 mm. and 2 mm., respectively.

At eleven days there is a further slight decrease in the number of

mitoses in all three series in both the former tongues and the old epithelium; absolutely the decrease is the smaller, the greater the number of mitoses is at nine days.

At fourteen days only at certain places a further very insignificant decrease takes place, while at other places the figures remain almost unchanged. At this time the number of mitoses is approximately the same in all three series, with the exception of the region of the former defect in the 4 mm. series where the number of mitoses is less than elsewhere. The number of mitoses is now approximately the same as in the normal skin. We may conclude that the differences in the rapidity in the outgrowth of the tongues and the resulting differences in the time of closure are mainly responsible for the differences in the variations in the mitoses in the different series. Closure of the wounds is followed by a sudden decline in the number of mitoses, especially in the area of the defect. Therefore the number of mitoses decreases earliest in the 4 mm. series, and absolutely we find there the smallest number of mitoses during the period over which our observations extended. We find therefore a greater number of mitoses in the smaller wound. Of course we have to take into consideration the fact that our observations extend only over an area of the old epithelium 1 mm. long. It is possible that in the 4 mm. series the increase in mitoses extends over a longer area than in the 2 mm. series.

TABLE VI.

No. of Mitoses in the Tongue or in the Epithelium Covering the Former Defect in an Area 1 Mm. Long x 50μ Wide.

Days.		
2	4 mm. series.	⅔
	2 " "	5
3½	4 " "	42
5	4 " "	5.1
	2 " "	40
7	4 " "	6
	2 " "	88
9	4 " "	6
	2 " "	30
11	4 " "	4.7
	2 " "	25
14	4 " "	4.8
	2 " "	21

We have so far considered the number of mitoses in the tongues and in the epithelium covering the former defect independently of the size of the epithelial area. If we reduce all the figures to an epithelial area 1 mm. long, in the same way that we did in the case of the old epithelium, the character of the curve is not changed considerably, but we find in the 2 mm. series the number of mitoses in the region of the old defect exceeding those in the old epithelium and remaining above the normal number for a longer period of time than the figures given above indicate (Table VI).

Changes in the Size of Cell and Nucleus.

The size of the cell and nucleus were measured at the point of insertion of the tongue. Two diameters of cell and of nucleus in the 4 and 2 mm. series are given in Tables II and IV (Columns 3 and 4). If we consider only two diameters, the changes in cell and nucleus are represented in Table VII.

TABLE VII.

	4 mm. series.		2 mm. series.	
	Cell.	Nucleus.	Cell.	Nucleus.
Days.				
Normal cell	75	49	75	49
2	147	88	119	67
3½	158	88		
5	117	59	145	81
7	124	64	142	75 (open).
			104	71 (closed).
9	114	64	118	68
11	115	56	88	60
14	109	54	81	62

We see that soon after the making of the wound the size of the cell and nucleus increase up to a maximum which is absolutely apparently only a little higher in the 4 mm. than in the 2 mm. series, which, however, is reached much earlier in the 4 mm. than in the 2 mm. series. The maximum is reached just before the closure of the wound in both series. After the closure a sudden decrease in the size of the cells takes place, and from that time on the size remains almost unchanged between the fifth and eleventh day in the 4 mm. series. At fourteen days a very slight decrease is observed, but the

size is still greater than in the normal epidermis. In the 2 mm. series a sudden decline takes place at the seventh day or soon afterwards, and from that time on the size of the cells continues to get smaller at a more rapid rate than in the 4 mm. series. But here also the cell size is still slightly above normal fourteen days after the making of the wound. The changes in the size of the nucleus follow a similar curve; but here the variations are smaller, and the deviations from the normal size not so great as in the case of the whole cell. On the whole, the curves indicating variations in cell and nuclear size, on the one hand, and in the number of mitoses run parallel to each other in the 4 mm. as well as in the 2 mm. series. But the size of cell and nucleus seems to return less quickly to the normal condition than the number of mitoses. Furthermore, the cell size increases more in the 4 mm. series than in the 2 mm. series, while the number of mitoses was more increased in the 2 mm. series.

Number of Rows of Living Cells and Width of Stratum Germinativum.

The last three columns in Tables I and III give the number of rows of living cells and the width of the stratum germinativum, at the tip of the tongue, insertion of tongue, and in the old epithelium near the insertion of the tongue. These figures are not so definite as those of the size of the cells, and in case there is an apparent contradiction between the two sets of figures, the figures for the size of the cells are to be preferred.

We see that at two days the number of cell rows is everywhere greater in the 4 mm. series than in the 2 mm. series, and the thickness of the stratum germinativum is also greater in the 4 mm. series. At three and one-half days the number of cell rows and the thickness of the stratum germinativum increase slightly. At five days the number of cell rows and the thickness of the living epithelium are still greater in both the 2 mm. and 4 mm. series; and the figures are similar in the 2 and 4 mm. series at the place of insertion of the tongues and in the old epithelium. The main difference exists in the epithelium covering the defect. In the 4 mm. series the two tongues have met at that time, exert a pressure on each other, and

we find, therefore, a very much thicker layer and more cell rows in the 4 mm. than in the 2 mm. series at this point. At seven days the figures are very similar to those at five days over the former defect in the 4 mm. series. From then until the fourteenth day a gradual decrease takes place in the thickness of the living epithelium as well as in the number of cell rows in this area.

In the 2 mm. series, on the other hand, we find the maximum in the number of cell rows and thickness of the living epithelium reached at the seventh day, again at the time of the closure of the wound. There the maximum is not so great as in the 4 mm. series. The push with which the two tongues coming from opposite sides meet each other is evidently greater in the 4 mm. series than in the 2 mm. series, and this push of the opposing tongues leads to the increase in the number of living cell rows and thickness of the epithelium in this area. Afterwards the epithelium evidently recedes again somewhat or is cast off. Between the eleventh and fourteenth day the decrease is greater in the 4 mm. series than in the 2 mm. series.

At the same time that the maximum is reached over the former defect, directly after the time of closure, a maximum is also reached at the point of insertion of the tongue and in the old epithelium in the 4 mm. series, while in the 2 mm. series the maximum at these two places is reached before the closure of the wound at the fifth day; from the direction of the old epithelium the push towards the wound extends into the tongue which leads to the subsequent closing. The maximum figures are very similar in the 2 and 4 mm. series. While, however, at the seventh day there is already a distinct diminution in the 4 mm. series, the decrease is less at seven days in the 2 mm. series at these two places; this is in accordance with the fact that at seven days the closure takes place in the 2 mm. series, while at that period the wound has already been closed for some time in the 4 mm. series. Afterwards in both series a gradual decrease takes place at these two areas. At the point of insertion the number of cell rows and the thickness of the epithelium are greater than in the old epithelium in both series, throughout the time of observation. From the eleventh to the fourteenth day the figures at these two places are very similar in the 4 mm. series to those in the 2 mm.

series. We may conclude that in both the 4 and in the 2 mm. series a push of the epithelium takes place in the direction towards the wound, and movements of the epithelium occur not only over the defect, but also in the neighboring epithelium, and that these movements may perhaps proceed in rows from the old epithelium towards the wound, so that a maximum is reached in the old epithelium at a slightly earlier period than over the defect. It thus appears that the primary process in the wound healing consists in movements of the epidermis towards the wound, that these movements are carried out with greater energy if the wound measures 4 mm. than if it measures 2 mm., that the pull of the epithelium calls forth mitotic cell division, and that pressure exerted by epithelial cells upon each other leads to a rapid diminution in the mitotic proliferation.

SUMMARY.

1. The larger the wound, the more rapidly the tongue enlarges and the earlier the closure of the wound takes place. Larger wounds heal, therefore, more quickly than smaller wounds within the variations in the size of the wound chosen in our experiments.

2. Both outgrowing of the tongues and contraction of the wound are concerned in the closing of the wound. A marked contraction sets in in the period preceding the closing of the wound and continues over a longer period with gradually diminishing intensity. The contraction, therefore, sets in earlier in the larger wounds. The contraction is also absolutely greater in the larger wound.

3. During wound healing the mitoses increase first markedly in the old epithelium and only very few mitoses can be found in the outgrowing epithelium during the first two days. Very soon the mitotic proliferation extends to the tongue and the number of proliferating cells may here become greater than in the old epithelium. With the closure of the wound a sudden fall in the number of mitoses takes place in both series. This fall is greatest in the tongue. Throughout the time of observation the number of mitoses is greater in the smaller wound. The fall in the number of mitoses directly after the closure of the wound is more sudden in the 4 mm. than in the 2 mm. series. It is, however, possible that the increase of mitoses extends over a larger area in the 4 mm. series than in the 2 mm. series.

4. It is probable that the difference in the rapidity in the outgrowth of the epidermal tongues and the resulting difference in the time of closure are mainly responsible for the difference in the variations in mitoses in the larger and smaller wounds. The longer the period of time over which the pull of the epithelium extends, the greater is the number of mitoses in this area. Therefore it is greater in the 2 mm. series. Closure of the wound is followed by a sudden decline in the number of mitoses especially in the area of the defect. Therefore the number of mitoses decreases earlier in the 4 mm. series, and we find here the smallest number of mitoses during the whole period of our observation.

5. The size of the epithelial cell and nucleus increases soon after the making of the wound. A maximum is reached in both the larger and smaller wound in the period just preceding the closure of the wound; this maximum is therefore reached earlier in the larger wound. Absolutely the maximal size reached in both kinds of wounds is approximately the same or only a little higher in the larger wounds. After the closure of the wound a sudden decline in the cell size takes place in the larger as well as in the smaller wounds. Then a more gradual decline sets in. Fourteen days after the operation the cells are still larger than in the normal skin. The variations in the size of the nucleus are similar to those in the whole cell, but less marked. The curves of variations in cell and nuclear size follow in both the larger and smaller wounds a curve similar to the variations in the number of mitoses. But the cell size returns more slowly to the normal condition than the number of mitoses.

6. The closure of the wound causes an increase in the number of epithelial rows over the defect. This increase is therefore reached at an earlier period in the larger wound. The increase is greater in the larger wound owing to the greater pressure which the two opposing cell layers exert upon each other in the larger wound. In the old epithelium the maximum in the number of cell rows is apparently reached slightly before the closure of the wounds. It seems that the epithelial movements leading to the closure of the wound start in the old epithelium and extend wave-like towards the wound.

7. It thus appears that the primary process in the wound healing consists in movements of the epidermis towards the wound, that

these movements are carried out with greater energy in the case of the larger wounds, that the pull of the epithelium calls forth mitotic cell division, and that pressure exerted by epithelial cells upon each other leads to a rapid diminution in the mitotic proliferation.

INTESTINAL OBSTRUCTION.

V. Proteose Intoxication.

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In earlier publications Whipple, Stone, and Bernheim (1, 2, 3, 4) have established facts which have an important bearing on this question of proteose intoxication. Some of these points will be reviewed, but for the detailed experiments the original papers should be consulted. In this work closed loops of intestine have been used because it has been established by previous experiments that an acute intoxication develops, and the toxic substance may be obtained in concentrated form in the material which accumulates in the closed loops. The intoxication noted in simple intestinal obstruction is similar, but usually less intense than that observed in dogs with closed or drained duodenal loops. Much evidence has been furnished to demonstrate that the essential toxic substance is very similar or even identical in obstruction and in closed intestinal loops.

All recent work speaks strongly for the theory of a definite intoxication in intestinal obstruction and closed loops of intestine. The doubts expressed by Hartwell and Hoguet (5) have been answered by Draper (6). Hartwell and Hoguet claimed that loss of fluid and consequent dehydration of the tissues were primary and the intoxication a secondary result of this dehydration. Whipple, Stone, and Bernheim have claimed that the intoxication was primary and the dehydration of the tissues secondary to the intoxication. Draper has been able to show that a dog can be dehydrated in four days by pilocarpin to the same degree encountered in duodenal obstruction. One of us has been able to bring about a similar dehydration and blood concentration by means of pilocarpin and vigorous salt purgation

over a period of thirty-six hours. The dogs which are dehydrated by means of drugs show no evidence of intoxication and recover their weight very promptly when given abundant food and water. It is safe to assume that there is a primary intoxication in intestinal obstruction and with closed intestinal loops.

When a loop of small intestine is washed out, closed by tapes, and a gastro-enterostomy made to reestablish the continuity of the intestine, we may attribute any resultant intoxication to one or both of two factors: (1) bacteria, and (2) intestinal mucosa. We have shown elsewhere that destruction of the mucosa prevents the formation of any toxic substance in the loop; in other words, the mucosa is essential to the production of the poison. Whether bacteria are essential or not is more difficult of proof and this point has not been established as yet beyond criticism. Murphy and Brooks (7) submit an experiment which suggests that bacteria are essential, but it may be claimed that the mucosa of a sterile drained loop, probably in a dog more or less strongly immunized, might not react in the usual manner. The poison formed in this inoculated loop did not give the usual reaction when given intravenously in a normal dog.

It has been claimed by Whipple, Stone, and Bernheim that the poison can be formed in a closed loop with no demonstrable lesions in the mucosa. This has been disputed by Hartwell and Hoguet (5, 8), and by Murphy and Brooks (7). It may be admitted that commonly the fluid accumulates in the closed loops, that this causes pressure and interference with the blood supply and finally injury to the mucosa. Some cases, however, are found in which only a little pasty material is found in a closed loop immediately after death. We wish to emphasize the fact that in such cases immediately after death we are unable to find the slightest histological evidence of injury to the mucosa. It is not necessary to find histological evidences of abnormality to support our belief that this mucous membrane is abnormal in that it forms a toxic proteose and allows its escape into the blood stream. We are convinced that these cells can present an abnormal physiological activity without any demonstrable histological change.

Absorption of this poison from the intestinal tract brings up some points of much practical importance. It is probable that injury of the mucosa will greatly facilitate toxic absorption, but it must be

remembered that the normal mucosa can not absorb any of the poison. Further, it is known that closed loops may be filled with much toxic material without giving any increase in intoxication. Dogs whose loops are filled with a lethal dose of toxin will live as long as control dogs whose loops are left empty at the end of the operation. Drained loops which are washed frequently give ample evidence of intoxication. This shows that much, if not the greater part of the intoxication is due to absorption from the mucous membrane alone rather than from the material in the lumen of the gut. Our attention should be concentrated on the mucous membrane in an effort to discover the fundamental change which is responsible for the toxin production and the reaction which may be responsible for its sudden return to normal activity.

The chemical nature of this poison is of great interest and of some practical importance. When its identity is established it will be possible to study all similar intoxications with the possibility of discovering the best means of combating the intoxication or neutralizing the poison or helping in its elimination. The fundamental objective of course is to check the formation of the poison at its source, but this may be impossible.

It is a very elusive substance and of necessity concealed in a mass of confusing material. Most workers in this field have hazarded a guess as to the identity of the poison. Albeck (9) believed that it belonged in the group of putrefactive poisons. Murphy and Brooks (7) believe that it may be similar to the substance sepsin isolated by Faust (10). It has been suggested that it might resemble the β-iminazolyl ethylamine of Dale (11). Some of the reactions of this closed loop poison resemble those produced by the anaphylatoxin.

As evidence for its chemical analysis and identification it is to be recalled that this substance is not capable of sensitizing guinea pigs for the characteristic anaphylactic reaction. Dogs can be immunized to the poison, but this protection is not great, and an immune animal can scarcely withstand a double lethal dose. The immunity does not reside in the body fluids which are inert toward the poison. The immune organs or the autolyzed tissue juices contain ferments which destroy the poison *in vitro* (Whipple, Stone, and Bernheim (3)).

It is to be recalled that this poison may be thrown out of a rich

albuminous solution by boiling. The poison is more or less completely removed by Berkefeld filters (Murphy and Vincent (12) and Kukula (13)). Our earlier experiments show that autolysis with normal intestinal mucosa for a week will not destroy the poison (2). Long autolysis with mucosa weakens its toxic power and after many months (8 to 12) the poison may be completely destroyed. This resistance to digestive enzymes is characteristic and of considerable importance.

EXPERIMENTAL OBSERVATIONS.

The experiments given below show that the poison is not destroyed by pancreatic digestion for seven days. The most important observation, that alcohol precipitates this poison, is of great help in the isolation of this substance. Five volumes of 95 per cent alcohol will cause complete precipitation of the poison, and the filtrate is free from the toxic substance. This point has been established beyond a doubt by repeated experiments, and in this way a great number of confusing substances may be eliminated. Further purification may be effected by solution of the alcoholic precipitate and reprecipitation by one-half saturation with ammonium sulphate. Dialysis may be used, as the toxin does not pass a collodion membrane. The pure substance is a primary proteose, and may be classed as a heteroproteose because it resists digestion. It is very toxic, and 100 mg. may be sufficient to poison a fifteen pound dog with the characteristic fatal reaction.

Methods.

Dogs were used in all the experiments. The kymograph observations were taken from the carotid artery exposed by a small incision at the root of the neck. In the abdominal operations all the usual aseptic precautions were observed. Ether anesthesia was used in all experiments. Hammarsten's method for testing the urine for proteoses may be outlined as follows. Equal parts of urine and saturated ammonium sulphate are heated to a boil. The precipitate is thrown down in a centrifuge, the supernatant fluid decanted, and the precipitate washed repeatedly with alcohol to remove the urobilin. The residue is dissolved in water, boiled, and filtered to remove albumin. The solution is extracted with chloroform and finally tested with the biuret.

Poison Not Destroyed by Complete Pancreatic Digestion.

Duodenal loop fluid (30 cc., a known lethal dose) was put away in the incubator at 38° C. after mixing with a freshly prepared dog's pancreas. The pancreas had been ground with sand, allowed to stand for 1 to 2 hours mixed with the loop fluid, made alkaline to litmus, and diluted to a thin soup. The mixture was protected from putrefaction by chloroform and toluol. Digestion proceeded for 7 days. There was no unpleasant odor, and the mixture was almost wholly fluid. It was passed through a thick Gooch crucible filter giving a clear amber fluid which was used to inject into a normal dog (Dog S-16).

Dog S-16.—Small adult mongrel, male; weight 14¼ pounds.

Feb. 17, 3 p.m. Intravenous injection of the clear filtrate just described (30 cc.). This gave the usual reaction seen after the injection of the toxic material into dogs. The initial fall in blood pressure was transient with rapid return to normal. The progressive lasting fall in pressure began at the end of 30 minutes. 3.30 p.m. Dog is shocked and shows air hunger. 8.00 p.m. Death with extremely low temperature.

Autopsy.—The findings are typical of death by a large dose of the poison found in closed duodenal loops. Splanchnic engorgement and fluid in the intestines are the striking features.

Poison Precipitated by Alcohol and Soluble in Water.

Duodenal loop fluid (40 cc.), precipitated by three volumes of 95 per cent alcohol and heated for an hour over a boiling water bath, was filtered.

1. The filtrate or alcoholic extract was evaporated slowly in a vacuum at 80° C. to a small volume and tested on a normal dog with negative results. Kymograph tracings taken during the injection showed a prompt initial fall in blood pressure with return to normal in one or two minutes. The substance which causes the initial fall in blood pressure similar to the reaction of tissue extracts is soluble in alcohol. The dog recovered without showing the least signs of intoxication.

2. The precipitate was extracted with ether for several days and the ether extract allowed to evaporate, giving a fatty residue. This ether residue was taken up in alcohol and tested on a normal dog with negative result.

3. The precipitate (extracted by alcohol and ether) was finally extracted with water over a water bath, and the watery filtrate obtained by rapid paper filtration. It was tested on Dog S-39. Fatal poisoning in three and one-half hours.

4. The residue was finally digested with intestinal mucosa effecting a complete solution. This fluid was tested on a normal dog with negative results.

Dog S-39.—Small yellow mongrel, male; weight 13¼ pounds.

Apr. 19, 11 a.m. Intravenous injection of watery extract of the alcoholic precipitate from the duodenal loop fluid. This caused some initial drop in blood pressure, followed by a return to normal lasting during the kymograph observa-

tion. 1.30 p.m. Dog is gravely shocked; much diarrhea and vomiting. 2.30 p.m. Death in collapse. Rectal temperature 30° C.

Autopsy.—The findings are typical of acute poisoning with duodenal loop fluid. Splanchnic congestion very marked. Blood concentration is striking; dry weight 26.3 per cent.

Alcoholic Precipitate Contains Poison.

Duodenal loop fluid (30 cc.) was allowed to digest with a little intestinal mucosa plus chloroform for over two weeks. This gave a clear broth-like filtrate which contains the poison in such experiments. This clear solution was treated with five volumes of 95 per cent alcohol, and the mixture was allowed to stand for three days after being brought to a boil over a water bath. A very scanty precipitate came down on standing. This precipitate was collected on a small filter and washed with alcohol and ether. It was estimated, by comparison with fibrinogen precipitates collected in similar manner, at 30 to 60 mg. This precipitate was completely dissolved in water and used for intravenous injection as before.

Dog O-74.—Small mongrel, female; weight 8½ pounds.

Apr. 21, 2.30 p.m. Intravenous injection of the above preparation caused no initial drop in blood pressure, but a slight secondary fall after a period of 30 minutes. 4 p.m. Dog vomits repeatedly. 5 p.m. Dog vomits repeatedly; salivation and diarrhea marked. 10 p.m. Death.

Autopsy.—Typical picture of acute poisoning by duodenal loop fluid.

The preceding experiments (Dogs S-39 and O-74) show that this poison can be obtained from a mixed solution by alcoholic precipitation. The precipitate when extracted with water yields all of the toxic substances, and the amount of the poison must be very small indeed.

Duodenal Loop Fluid—Toxic Protease.

Loop fluid (1,100 cc.) was collected from six dogs. All these dogs had closed loops of varying length including the duodenum and upper part of the jejunum, varying from 20 to 30 inches in length. The loops were isolated by means of tapes which occluded the lumen of the intestine, and a gastro-enterostomy was performed a few inches below the lower ligature. This mixture was preserved a few days with chloroform and toluol at room temperature. It was then incubated for 48 hours at 38° C. and again allowed to stand at room temperature for 2 weeks.

1,000 cc. of the mixture were precipitated with 5 liters of 95 per cent alcohol at room temperature and allowed to stand at room temperature for many weeks with occasional shaking.

100 cc. were tested by preliminary experiments to determine the amount of toxic substance and some of its reactions. The fluid was boiled over a free flame giving a moderately voluminous precipitate. The supernatant fluid was poured

off and the coagulum extracted again with boiling water which was added to the decanted fluid, making about 120 cc. in all. This fluid was filtered through paper. It reacted faintly alkaline to litmus. Half saturation with ammonium sulphate gave a moderate flocculent precipitate. Five volumes of 95 per cent alcohol gave about the same amount of precipitation. Complete saturation with ammonium sulphate gave but a little more precipitate than did half saturation. Saturation with sodium chloride gave less precipitate than alcohol or ammonium sulphate.

Dog 15–21.—Normal adult spaniel, female; weight 23 pounds.

Mar. 31, 11 a.m. Intravenous injection of 100 cc. of the clear duodenal loop fluid which had been tested above. Temperature at end of the injection 99.4° F. 12 m. Dog not intoxicated, vomited little mucus. No feces passed. Rectal temperature 99.7°F. 12.30 p.m. Dog is vomiting again and passed one semisolid stool; pulse is fair. 1.30 p.m. Dog is vomiting again but is not severely shocked. 3 p.m. Dog is quite sick. Pulse is of a low tension. Dog vomited bile-stained mucus. Temperature 102.5° F. Next day dog appeared normal.

This experiment shows that one-eleventh of the entire fluid did not contain a lethal dose of poison and probably contained about one-half or less than one-half of a lethal dose. We may assume with some justification that the remaining 1,000 cc. of duodenal loop fluid contained from 3 to 6 times a lethal dose for a dog of 15 to 20 pounds' weight.

Duodenal loop fluid (1,000 cc.) was precipitated with 5 liters of 95 per cent alcohol, standing at room temperature for 12 weeks. The precipitate was not abundant. It was thrown upon a paper filter and partially dried. The precipitate was then washed from the filter paper with about 550 cc. of hot distilled water. The mixture made faintly acid to litmus with acetic acid and boiled over the free flame gave an abundant flocculent precipitate which was removed by centrifugalization. The supernatant fluid was poured through a paper filter, giving a total amount of 350 cc. of pale canary yellow, slightly opalescent fluid.

Of this clear filtrate 250 cc. were precipitated with an equal volume of saturated solution of ammonium sulphate and allowed to stand at room temperature. The abundant precipitate appeared slowly and was finally thrown down by the centrifuge. The supernatant fluid was decanted and the precipitate dried between filter paper. This material was dissolved in water to about 125 cc. and heated to boiling over a free flame. It gave a fine flocculent precipitate which was thrown down in the centrifuge, and amounted to about 0.25 the volume of the original ammonium sulphate precipitate. This represents almost the last trace of albumin. The supernatant fluid is almost water-clear and is faintly acid to litmus, but it gives a good precipitate on half saturation of ammonium sulphate. The volume of this solution equals 115 cc., and 65 cc. of this fluid given intravenously in Dog 15–50 cause fatal intoxication in 3½ hours (see also Dog 15–51).

Dog 15–50.—Small black and tan, adult, male; weight 13 pounds.

June 11, 12 m. Kymograph observation with ether anesthesia. Purified loop fluid (65 cc.), described above, given intravenously. Blood pressure was very little influenced by this injection, but the heart was somewhat slowed and respira-

tion was considerably accelerated. Muscular twitchings were quite conspicuous at the end of the injection and this was due probably to the ammonium sulphate remaining in this solution. 12.45 p.m. Dog was removed from kymograph and out of ether. Passed one soft stool and vomited once. 2.30 p.m. No vomiting, but considerable salivation; pulse is weak and dog is cold and looks badly shocked. 3.15 p.m. Blood pressure very low; no vomiting and no diarrhea. Dog in semi-stupor and very cold. 3.45 p.m. Condition the same; temperature 96.8° F. 4 p.m. Death with autopsy immediately.

Autopsy.—Blood examination showed a considerable excess of antithrombin, as is usual after injection of the crude duodenal material; thorax and the lungs are normal; liver is deep purple and remarkably engorged, otherwise normal. Spleen is greatly enlarged and the splanchnic vessels are very conspicuous. Stomach shows a pale pylorus and cardia and a deep pinkish mucosa in the middle zone. Mucus and fluid very abundant in stomach and small intestine. Duodenum shows a deep purple red velvety mucosa coated with large amounts of mucus and contains much fluid. This color fades to a pink color in the ileum. The colon shows a mottled pinkish red mucosa. This picture is typical of acute poisoning by a large dose of duodenal loop fluid or fluid obtained from above an intestinal obstruction.

The remaining purified duodenal fluid (50 cc. in amount) which had been precipitated with alcohol and then with ammonium sulphate, dissolved in water, boiled, and filtered, was next dialyzed against 0.8 per cent sodium chloride in a collodion tube. This gave a turbidity and slight increase in volume. After dialysis for 18 hours the fluid was boiled and a small precipitate removed by the centrifuge. Filtration through paper gave an odorless, water-clear, but slightly opalescent fluid which was used for further tests. Dialyzed fluid (5 cc.) was precipitated in an Esbach tube with Tsuchiya's reagent (phosphotungstic acid 1.5, hydrochloric acid 5, alcohol 100 parts). This gave 1 gm. per 1,000 cc., as read from the tubes. This represents about 1 mg. per cc. of fluid. The dried weight was estimated carefully for 2 cc. and equalled 1.04 per cent. Nitrogen determination showed that the ammonium sulphate had not been completely removed by dialysis. The dried weight by calculation, allowing for 0.8 per cent sodium chloride, gives a maximum of 120 mg. of material in 50 cc. of this solution. The Esbach determination gives 50 mg. per 50 cc. The first estimation by the dried weight is probably too high, but more accurate than the Esbach. A fairly safe estimate of 100 mg. of proteose in this 50 cc. of fluid may be made. This fluid (50 cc.) was injected into Dog 15–51 with fatal result.

Dog 15–51.—Small fox-terrier, female; weight 15½ pounds.

June 14, 12 m. Kymograph observation with ether anesthesia. Solution of pure proteose (50 cc.) from duodenal loop fluid given intravenously. This caused no reaction on the blood pressure or pulse beat. 12.30 p.m. Animal seems quite unaffected by injection. Removed from kymograph. 1.30 p.m. Dog recovered and seems normal; walks about cage. 4 p.m. Dog is prostrated and greatly shocked; slow deep respiration, vomiting, and diarrhea noted. Temperature 104.2° F. Pulse tension poor. 10 p.m. Dog appears shocked and cold.

June 15, 9 a.m. Dog found dead and cold; much fluid feces in cage.

Autopsy.—Thorax, heart, and lungs normal; spleen and liver only moderately congested; stomach contains blood-tinged fluid and shows moderate congestion of the mucosa. Duodenum contains a good deal of mucus and fluid, and the mucosa is pinkish red and definitely engorged with blood. Jejunum and ileum show a similar picture and contain much watery fluid with mucus. This picture is characteristic of moderately acute intoxication and this dog was given just about a minimal lethal dose of the poison, causing death in about 15 hours with characteristic delayed symptoms of shock, associated with vomiting and diarrhea. It is probable that this dog received approximately 100 mg. of purified proteose obtained from duodenal loop fluid.

This isolation of a primary proteose from the duodenal loop fluid is well established by the above experiments. One liter of duodenal loop fluid contained several lethal doses of the characteristic poison. The toxic substance was first precipitated by five volumes of 95 per cent alcohol and the precipitate dissolved in water. This solution made faintly acid and boiled gave an abundant albuminous precipitate which was removed. The solution was treated with equal parts of a saturated solution of ammonium sulphate which gave a white flocculent precipitate. This precipitate was dissolved in water and the solution again boiled to remove all albumin. This clear filtrate contained the characteristic poison, and 65 cc. poisoned a dog fatally in four hours (Dog 15–50). The rest of this clear filtrate was dialyzed for eighteen hours, again boiled and filtered, giving a water-clear, slightly opalescent fluid. This fluid (50 cc.) contained about 100 mg. of proteose and was sufficient to poison fatally a dog weighing fifteen pounds (Dog 15–51).

This method of isolation removes practically all substances from the duodenal loop fluid mixture with the exception of the primary proteoses. This pure substance gives the identical toxic symptoms noted after injection of the crude duodenal loop fluid. Moreover, the alcoholic extract or filtrate is non-toxic and the bulk of the toxic material can be isolated and purified without great loss. This is strong, if not conclusive, evidence that the essential toxic substance has been isolated from the duodenal loop fluid and that the toxic substance is a primary proteose.

DISCUSSION.

Many interesting points concerning proteose intoxication have been brought out by the work of Chittenden (14) and his coworkers. These investigators show that certain proteoses may stop urinary secretion without much blood pressure reaction. This toxic proteose of intestinal obstruction may cause a sudden stoppage in urinary secretion and no primary blood pressure effect. The fall in blood pressure may not appear until hours after the injection when the symptoms of shock are in evidence.

Some proteoses may be changed before their excretion in the urine,—a primary proteose being excreted as a secondary proteose. It is very important to emphasize that when these proteoses escape from the body they do so by way of the kidneys. It has been shown by Hartwell and Hoguet (5) and others that administration of fluid is of benefit in the intoxication of intestinal obstruction, and it is highly probable that the diuresis aids in the elimination of the poison which may escape in part by way of the urine.

We have attempted to isolate this toxic proteose from the urine of dogs with intestinal obstruction and closed loops of intestine, and following injection of the toxic proteose. There are obvious difficulties in this work when we consider the small amount of the poison (100 mg.) which may produce fatal intoxication. Its injection in considerable amount is attended by cessation of renal excretion. It is not sufficient to state that a proteose appears in the urine after the intravenous injection of this toxic proteose or after an experimental closed intestinal loop. Various procedures may cause albuminuria in a dog, and this is often associated with a definite proteosuria (as shown by Hammarsten's method) without any evidences of intoxication. The determination of a proteose in small amounts in a dog's urine has no special significance. We have attempted to isolate a toxic proteose from dog's urine after obstruction or closed intestinal loops, but so far without success. It is possible that this specific primary proteose may be modified in some way before its excretion in the urine.

Animals react differently to the proteose groups of poisons. Underhill (15) and others point out that dogs are susceptible to pro-

teose intoxication, whereas cats and rabbits are very resistant. Davis and Morgan (16) have shown that cats are very resistant to the poison found in the closed intestinal loops of dogs or cats. Moreover, cats will survive a closed intestinal loop of a certain type much longer than a dog under parallel conditions.

Dogs react constantly to this proteose obtained from closed intestinal loops. The intensity of the clinical symptoms of salivation, vomiting, diarrhea, and prostration depend upon the amount of poison injected. The blood may show a great increase in antithrombin, which will delay or completely prevent clotting *in vitro*. This reaction is variable, but usually follows intravenous injection of a variety of proteoses. In fatal poisoning the splanchnic engorgement is the striking feature. The liver and spleen are swollen and purple. The mucosa of the duodenum especially, but also of the whole intestine, is greatly congested and may be a velvety purplish red color.

It is to be emphasized that other poisons can cause this poisoning with peculiar splanchnic paralysis and engorgement in dogs. It has been noted with various putrefactive poisons, toxic bases or amines (Faust), also after large doses of adrenalin, and in fatal anaphylaxis. It is not safe to draw conclusions about the nature of a given poison solely from its physiological action, but chemical tests must be added.

An interesting experiment has been performed by Murphy and Brooks (7), who isolated the gall bladder after introducing diluted intestinal contents. The dogs died with symptoms of intoxication, and the contents of their gall bladders were toxic to normal dogs, giving the usual picture of proteose intoxication. It is possible that growth of bacteria in association with the mucosa of the gall bladder can produce a toxic proteose. More work must be done, however, to establish this important point. On the other hand, it is possible that some putrefactive poison is responsible, because such poisons and toxic proteoses give similar reactions when given intravenously in dogs.

It is to be emphasized that the group of putrefactive poisons is not found in closed intestinal loops, and the only toxic element is a primary proteose.

Proteose intoxication causes a striking rise in the incoagulable nitrogen of the blood, which may double in amount in a period of three hours. Intestinal obstruction or a closed intestinal loop may cause a great rise in the non-coagulable blood nitrogen, which may even rise to ten times normal. This seems to depend within limits upon the severity and acuteness of the intoxication. Detailed experiments will be published in the near future, and the incoagulable nitrogen may prove to be of clinical value from the standpoint of diagnosis and prognosis.

SUMMARY.

A definite intoxication develops as a result of a closed intestinal loop and toxic material accumulates in the closed loops. Much evidence has been submitted to show that this loop poison causes the intoxication observed after producing a closed intestinal loop. Sufficient evidence has been presented to prove that the essential poison is present in these closed intestinal loops, and usually in concentrated form.

Chemical study of the contents of closed intestinal loops shows that a single substance or group of substances possesses toxic properties. This resists autolysis and pancreatic and ereptic digestion. It is thrown out of solution by five volumes of alcohol or by half saturation with ammonium sulphate. It is readily soluble in water and is not injured by boiling. It is not removed by dialysis. The method of isolation excludes practically all substances except primary proteoses. The characteristic resistance to digestive enzymes suggests a heteroproteose.

Proteose intoxication in dogs gives a picture identical with that described after poisoning with intestinal loop fluid: early salivation and vomiting, followed by diarrhea and prostration, fall in temperature and blood pressure, and finally death in collapse. Autopsy shows essentially a splanchnic paralysis and remarkable engorgement of liver and spleen, but especially of the mucosa of the duodenum and small intestine. The blood shows great concentration due to loss of fluid and may remain incoagulable because of an excess production of antithrombin.

Proteoses escaping from the blood are excreted in the urine. This toxic proteose concerned in intestinal obstruction has not yet been isolated in the urine, but may be excreted by the kidneys. This probably explains the clinical improvement and lessened intoxication noted after transfusion.

Experimental evidence points to a primary proteose as the essential poison concerned in the intoxication of closed intestinal loops and intestinal obstruction.

BIBLIOGRAPHY.

1. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *Jour. Exper. Med.*, 1913, xvii, 286.
2. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *Jour. Exper. Med.*, 1913, xvii, 307.
3. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *Jour. Exper. Med.*, 1914, xix, 144.
4. Whipple, G. H., Stone, H. B., and Bernheim, B. M., *Jour. Exper. Med.*, 1914, xix, 166.
5. Hartwell, J. A., and Hoguet, J. P., *Jour. Am. Med. Assn.*, 1912, lix, 82.
6. Draper, J. W., *Jour. Am. Med. Assn.*, 1914, lxiii, 1079.
7. Murphy, F. T., and Brooks, B., *Arch. Int. Med.*, 1915, xv, 392.
8. Hartwell, J. A., Hoguet, J. P., and Beekman, F., *Arch. Int. Med.*, 1914, xiii, 701.
9. Albeck, V., *Arch. f. klin. Chir.*, 1902, lxv, 569.
10. Faust, E. S., *Arch. f. exper. Path. u. Pharmakol.*, 1904, li, 248.
11. Dale, H. H., and Laidlaw, P. P., *Jour. Physiol.*, 1910–11, xli, 318.
12. Murphy, F. T., and Vincent, B., *Boston Med. and Surg. Jour.*, 1911, clxv, 684.
13. Kukula, *Arch. f. klin. Chir.*, 1901, lxiii, 773.
14. Chittenden, R. H., Mendel, L. B., and Henderson, Y., *Am. Jour. Physiol.*, 1898–99, ii, 142.
15. Underhill, F. P., *Am. Jour. Physiol.*, 1913, ix, 345.
16. Davis, D. M., and Morgan, H. S., *Bull. Johns Hopkins Hosp.*, 1914, xxv, 39.

ICTERUS.

A Rapid Change of Hemoglobin to Bile Pigment in the Pleural and Peritoneal Cavities.

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In an earlier communication (1) we have been able to show that bile pigment could be formed from hemoglobin without the agency of the liver. Solutions of hemoglobin were introduced into the blood vessels of dogs whose livers had been excluded from any part in this reaction. There was a prompt formation of bile pigment from hemoglobin with no possible direct liver action. This transformation can take place within a space of two hours when active circulation is maintained in the head and thorax alone. It seemed probable that the endothelium might be the tissue whose activity was responsible for this change of hemoglobin to bile pigments.

This work has received confirmation from experiments of McNee (2) who repeated the experiments of Minkowski and Naunyn with geese. He found that icterus did develop without liver activity and found evidences of endothelial activity. He suggests (3) that the Kupffer cells may normally transform hemoglobin to bile pigments.

The experiments given below prove conclusively that still other tissues can rapidly transform the hemoglobin pigment into bile pigment. There can be no question of any direct liver activity in these experiments. Some transformation may take place within eight hours, but the pigment production in twenty-four hours can usually be estimated with considerable accuracy. This mesothelium of the serous cavities can not effect this transformation as promptly as it is effected in the circulating blood, but the contact with the living pleural cells is not as active and intimate,—not a circulatory contact.

If we assume that the capillary endothelium can transform hemoglobin to bile pigment, it is at once obvious how intimate is the contact of the circulating hemoglobin solution with the living protoplasm of the vessels. The solution of hemoglobin in the serous cavities may be in more or less motion (respiration, peristalsis, or body movements), but at best it is not in very intimate contact with the living protoplasm. The wonder is that the transformation is so prompt and easily recognizable. It is impossible to estimate the relative activity of endothelium and mesothelium, but there can be little difference if we allow for the rapidity of circulation and surface contact.

It has been claimed by older workers (Virchow (4) and many others) that blood standing long in contact with living tissues was slowly changed to a golden pigment hematoidin which is chemically equivalent to bilirubin. Virchow used the term hemolytic icterus for this reason. Guillain and Troisier (5) noted in human cases with pleural hematomas that bile pigment was formed after a considerable period with no complicating liver abnormality. Von der Bergh and Snapper (6) were able to find greater amounts of bile pigment in serous exudates than in the blood serum, and they speak in favor of the extrahepatic formation of bile pigment. That the serous cavities can rapidly transform hemoglobin to bile pigment has not been hitherto recognized.

EXPERIMENTAL OBSERVATIONS.

The experiments are not all given in detail, but the essential facts are given in Tables I and II. A typical experiment from each group is given in sufficient detail, and it is to be emphasized that the majority of these experiments caused no inflammation or even permanent injury to the pleural or peritoneal cavity. In a few cases due to slips in technique there developed a pleurisy or a peritonitis, but these complications did not modify the transformation of hemoglobin to bile pigment. The crystalline hemoglobin causes a slight irritation in the pleural or peritoneal cavity, and it is possible that the inflammatory reaction may assist in the transformation, but it is not an essential factor.

Method.

Active and vigorous dogs were used in all of our experiments. The urine was obtained by catheter, and in every case it was examined before the experiment. The Huppert-Salkowski test for bile pigments was used in examining the urine, and unless otherwise stated 20 cc. of urine were employed in the test. Either fresh or crystalline dog hemoglobin was used. The fresh hemoglobin was obtained by laking washed erythrocytes with distilled water.

The preparation of crystalline hemoglobin was carried out according to the method of Bradley and Sansum (7). The red blood cells obtained from defibrinated dog blood by centrifugalization were washed eighteen or twenty times with normal salt solution, then they were laked with toluene and a little distilled water. The toluene layer was removed and the solution centrifugalized and decanted from the stroma material. This hemoglobin solution was then mixed with 20 per cent by volume of toluene, shaken thoroughly, and set aside in the cold. After twenty-four or forty-eight hours the mixture is nearly a solid mass of large well formed crystals. The crystals are collected by centrifugalization, washed with cold water, spread thin on glass, and dried in a stream of warmed air. Fresh 0.6 per cent salt solution was used as a diluting medium for the fresh or crystalline dog hemoglobin.

The fluids were introduced into or withdrawn from the pleural or peritoneal cavity through a trocar. After withdrawing the pleural or peritoneal fluids they were centrifugalized immediately, the supernatant fluid was decanted and made definitely alkaline by the addition of a saturated solution of sodium carbonate, then a 10 per cent solution of calcium chloride was added until precipitation was complete. After centrifugalization at high speed the supernatant fluid was again poured off, and the calcium bile pigment compound remained as a yellowish precipitate. The precipitate was washed free from other coloring matter and collected again by centrifugalization. Finally the precipitate dissolved in a hot solution of 5 per cent hydrochloric acid in 95 per cent alcohol gave the characteristic blue green color when bile pigments were present.

The method used to determine the amounts of bile pigments quantitatively will be published in the near future. This method in abstract consists of reading the blue green color of the acid alcohol extract in a fixed dilution against a permanent wedge of similar color which has been standardized against chemically pure bilirubin solutions of known amounts. The common sulphonephthalein colorimeter may be used to advantage.

Pleural Experiments.

Bile Pigments Formed from Hemoglobin in Periods of 8 to 66 Hours.

Dog 15–11.—8 and 24 hours. (Table I.) Short haired mongrel, male; weight 14 pounds.

Mar. 17. Dog is normal. 9.30 a.m. 20 cc. of concentrated urine are negative for bile pigments. 10 a.m. 20 cc. of washed, laked red blood cells are introduced into the right pleural cavity with 500 cc. of sterile 0.6 per cent salt solution. 11.10 a.m. No respiratory embarrassment. 2 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 6 p.m. 100 cc. of dark red fluid removed from right pleural cavity are negative for bile pigments. 20 cc. of urine are negative for bile pigments and hemoglobin.

Mar. 18, 9.35 a.m. Dog is very active. Rectal temperature 39.1° C. 20 cc. of urine negative for bile pigments and hemoglobin. 10 a.m. 158 cc. of dark red fluid recovered from right pleural cavity are positive for bile pigments. 11.20 a.m. Right pleural cavity irrigated with 500 cc. of 0.6 per cent salt solution. Appetite is fairly good.

Mar. 19, 9 a.m. Dog is listless. Rectal temperature 40.2° C. Respiratory movements over right thorax are impaired.

Mar. 20, 10 a.m. Rectal temperature 39.1° C. Dog is very active and eats well. Respiration normal.

Dog 15–34.—8 hours. (Table I.) Mongrel setter, male; weight 55 pounds.

May 12. Dog is active and vigorous. 8.30 a.m. 20 cc. of urine are negative for bile pigments. 8.45 a.m. 500 cc. of water are introduced into the stomach through a stomach tube. 9.30 a.m. 30 cc. of washed, laked red blood cells are introduced into the right pleural cavity with 1,500 cc. of sterile 0.6 per cent salt solution. 11 a.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 5 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 5.30 p.m. 1,175 cc. of dark red, turbid fluid recovered from right pleural cavity. Chemical test is positive (suspicious) for bile pigments. 5.50 p.m. Right pleural cavity is irrigated with 500 cc. of 0.6 per cent salt solution. 6 p.m. Drinks some milk.

May 13, 9.30 a.m. Dog is listless and refuses to eat. Rectal temperature 102.3° F. Respiratory movements over right thorax are impaired, and a friction rub is palpable.

May 14, 10 p.m. Very active. Rectal temperature 101.8° F. Respiratory movements are normal.

Dog 15–39.—17 and 66 hours. (Table I.) Black mongrel, female; weight 32 pounds.

May 10. Dog is in excellent condition. 9.30 a.m. 20 cc. of concentrated urine are negative for bile pigments. 11.15 a.m. 30 cc. of washed, laked red blood cells suspended in 1,100 cc. of sterile 0.6 per cent salt solution are introduced into the right pleural cavity. 5.20 p.m. 930 cc. of dark red fluid, recovered from the right pleural cavity, give a positive (suspicious) test for bile pigments. 20 cc. of urine are negative for bile pigments and hemoglobin. 5.30 p.m. Right pleural cavity is irrigated with 500 cc. of 0.6 per cent salt solution. 6 p.m. Respiratory movements over right thorax are somewhat delayed.

May 11. Dog is active and vigorous. Rectal temperature 102.6° F. Respiratory movements are normal.

May 17. Dog is in excellent condition. Weight 32 pounds. 3.30 p.m. 20 cc. of urine are negative for bile pigments. 5 p.m. 30 cc. of washed, laked red blood cells are introduced into the left pleural cavity with 1,000 cc. of 0.6 per cent salt solution. 6.10 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. Respiratory movements are somewhat delayed over left thorax.

May 18, 9.30 a.m. Rectal temperature 103.2° F. Respiratory movements are markedly delayed over left thorax. 20 cc. of urine are negative for bile pigments and hemoglobin. 10 a.m. 390 cc. of dark red fluid recovered from the left pleural cavity contain 0.14 mg. of bile pigment. 5.30 p.m. Animal is quite active.

May 19. Dog is not very lively. 9 a.m. Rectal temperature 102.8 °F.

May 25. Weight 32 pounds. Dog is active and vigorous. Rectal temperature 102.2° F. 4.30 p.m. 20 cc. of urine are negative for bile pigments. 4.45 p.m. 500 cc. of water are introduced into the stomach through a stomach tube. 4.50 p.m. The right pleural cavity is irrigated with 500 cc. of sterile 0.6 per cent salt solution. The fluid recovered is water-clear; bile pigment test negative. 6.10 p.m. 10 gm. of crystalline dog hemoglobin dissolved in 1,100 cc. of 0.6 per cent salt solution are introduced into the right pleural cavity. 6 p.m. Respiratory distress is not marked.

May 26. Dog is quite active and eats well. The movements of the right thorax are somewhat impaired. Rectal temperature 101.8° F. 2.10 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin.

May 27. Rectal temperature 102.2° F. Movements of the right thorax are markedly impaired. Refuses food. 12.35 p.m. 20 cc. of urine are faintly positive for bile pigments.

May 28. Rectal temperature 102.8° F. 10.30 p.m. 20 cc. of urine are faintly positive for bile pigments. 11 a.m. 510 cc. of dark red turbid fluid recovered from the right pleural cavity contain 5.40 mg. of bile pigments. 11.20 a.m. Right pleural cavity is irrigated with 500 cc. of sterile 0.6 per cent salt solution.

May 29. Dog is normal.

Dog 15–52.—24 hours. (Table I.) Bull terrier mongrel, male; weight 41.5 pounds.

June 16. Dog is normal. 10 p.m. 20 cc. of urine are negative for bile pigments. 11.35 a.m. 9 gm. of crystalline dog hemoglobin dissolved in 1,000 cc. of 0.6 per cent salt solution are injected into the right pleural cavity. 12 m. Respiratory distress is not marked. 6 p.m. Animal drinks some milk.

June 17. Dog is quite active. Respiratory movements over right thorax are impaired. Rectal temperature 102.8° F. 10 a.m. 20 cc. of urine are negative for bile pigments. 11.30 a.m. 575 cc. of dark red, turbid fluid recovered from the right pleural cavity contain 0.18 mg. of bile pigment. 5 p.m. Respiratory movements are quite normal.

June 18. Dog is very active. Rectal temperature 102.4° F.

TABLE I.

Hemoglobin Changed to Bile Pigment in Pleural Cavity.

Dog No. Date.	Weight.	Pleural cavity.	Time in pleural cavity.	Bile pigment tests.	Bile pigments.	Fluid introduced.	Fluid recovered.	Bile in urine. Before.	Bile in urine. After.	Remarks.
1915	*lbs.*		*hrs.*		*mg.*	*cc.*	*cc.*			
15–11 Mar. 17	14.0	Right.	8	0	0	620	100	0	0	Fresh dog hb.
15–11* Mar. 17	14.0	"	24	+	—	620	158	0	0	" " "
15–34 May 12	55.0	"	8	(?)+	—	1,580	1,175	0	0	" " "
15–39 May 10	32.0	"	7	(?)+	—	1,160	930	0	0	" " "
15–40 May 10	22.0	"	6	(?)	—	700	555	0	0	" " "
15–41 May 12	36.0	"	8	(?)+	—	1,480	1,230	0	0	" " "
15–39 May 17	32.0	Left.	17	+++	0.14	1,105	390	0	0	" " "
15–40 May 17	21.5	"	18	+++	0.09	805	265	0	0	" " "
15–52 June 16	41.5	Right.	24	++++	0.18	1,000	575	0	0	Crystalline dog hb., 10 gm.
15–54 June 16	36.5	"	24	++	—	800	395	0	0	" " " 7 "
15–53 June 16	29.5	"	25	+	—	800	408	0	0	" " " 7 "
15–56 June 16	39.0	"	25	++	—	900	318	0	0	" " " 7 "
15–41 May 20	36.5	Left.	43	+++	0.15	805	254	0	+ (faint)	Fresh " "
15–43 May 20	34.0	Right.	44	++++	0.36	1,100	808	0	0	Crystalline " " 4 "
15–40 May 25	22.0	"	65	++++	3.60	940	430	0	+ (faint)	Fresh " "
15–39* May 25	32.0	"	66	++++	5.40	1,100	510	0	+ (faint)	Crystalline " " 10 "

*See history for details.

Dog 15–43.—43 hours. (Table I.) Mongrel spaniel, male; weight 34 pounds.

May 20. Dog is in excellent condition. Rectal temperature 102° F. 12.10 p.m. 20 cc. of urine are negative for bile pigments. 12.15 p.m. 500 cc. of water are introduced into the stomach through a stomach tube. 3.10 p.m. 4 gm. of crystalline dog hemoglobin dissolved in 1,100 cc. of sterile 0.6 per cent salt solution are introduced into the right pleural cavity. 5.30 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin.

May 21. Dog is active and eats well. Respiratory movements over the right thorax are impaired. Rectal temperature 102.2° F.

May 22. Rectal temperature 103.1° F. 10.30 a.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 11 a.m. 808 cc. of dark red, turbid fluid recovered from the right pleural cavity contain 0.36 mg. of bile pigments.

May 23. Rectal temperature 101.8° F. Dog is normal.

The preceding experiments are conclusive proof that mesothelium can rapidly transform hemoglobin into bile pigment. The lining cells of the pleural cavity like other body cells (probably endothelium) can form bile pigments out of hemoglobin, either freshly laked or crystallized. This solution of hemoglobin is not in very intimate contact, not in circulatory contact, as is hemoglobin in contact with the endothelium of the capillaries, yet the transformation takes place promptly in a few hours. There is experimental evidence of this bile pigment transformation in the pleura within eight hours, but a very definite amount is formed within eighteen to twenty-four hours, an amount often sufficient for accurate determination.

Dog 15–20.—8 hours. (Table II.) Mongrel poodle, male; weight 19.5 pounds.

Mar. 29. Dog is normal. 10 a.m. 20 cc. of concentrated urine are negative for bile pigments. 10.30 a.m. 35 cc. of washed, laked red blood cells are introduced into the peritoneal cavity with 800 cc. of 0.6 per cent salt solution. 12.30 a.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 2 p.m. Rectal temperature 101.3° F. 5.30 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 6.30 p.m. 300 cc. of dark red turbid fluid recovered from the peritoneal cavity give a questionably positive test for bile pigments. 6.45 p.m. Peritoneal cavity is irrigated with 2,000 cc. of 0.6 per cent salt solution. Urine gives positive (suspicious) test for bile pigments.

Mar. 30. Dog is very lively and eats well. Rectal temperature 101.7° F.

Dog 15–43.—28 hours. (Table II.) Mongrel spaniel, male; weight 33.5 pounds.

June 17. Dog is in excellent condition. 9.45 a.m. 20 cc. of urine are negative for bile pigments. 11.15 a.m. 7 gm. of crystalline dog hemoglobin dissolved in 2,800 cc. of 0.6 per cent salt solution, introduced into the peritoneal cavity.

12 m. Dog shows no embarrassment. 5.30 p.m. Animal listless and refuses to eat. Considerable rigidity of abdomen.

June 18. Rectal temperature 103.8° F. Animal is listless, will not eat, and lies quietly in cage. Abdominal muscles are very tense. 2 p.m. 20 cc. of urine are positive for bile pigments. 3.20 p.m. 755 cc. of dark red turbid fluid recovered from the peritoneal cavity give a positive test for bile pigments. 3.35 p.m. Peritoneal cavity irrigated with 3,000 cc. of sterile 0.6 per cent salt solution. 5 p.m. Rectal temperature 104.2° F.

June 19. 9 a.m. Animal is found dead. Autopsy showed a diffuse fibrinous peritonitis. Other organs are negative.

Dog 15–55.—48 hours. (Table II.) Mongrel hound, male; weight 40.5 pounds.

June 16. Dog is in excellent condition. 2 p.m. 20 cc. of urine are negative for bile pigments. 2.40 p.m. 7 gm. of crystalline dog hemoglobin dissolved in 3,000 cc. of 0.6 per cent salt solution introduced into the peritoneal cavity. 6 p.m. Dog is quite active. 20 cc. of urine are negative for bile pigments and hemoglobin.

June 17. Rectal temperature 102.2° F. 10.10 a.m. 20 cc. of urine are positive for bile pigments. Hemoglobin negative.

June 18. Dog is very lively and eats well. 9.30 a.m. Rectal temperature 103.6° F. 1.30 p.m. 20 cc. of urine are negative for bile pigments and hemoglobin. 2.30 p.m. 310 cc. of dark red, turbid fluid recovered from peritoneal cavity contain 0.27 mg. of bile pigment. 2.50 p.m. Peritoneal cavity irrigated with 2,000 cc. of 0.6 per cent salt solution. 5.30 p.m. Dog is very active.

June 30. Dog is normal.

TABLE II.

Hemoglobin Changed to Bile Pigment in Peritoneal Cavity.

Dog No. Date.	Weight.	Time in peritoneal cavity.	Bile pigment tests.	Bile pigments.	Fluid introduced.	Fluid recovered.	Bile in urine.		Remarks.
							Before.	After.	
1915	*lbs.*	*hrs.*		*mg.*	*cc.*	*cc.*			
15–20									
Mar. 29	19.5	8	(?)	—	985	300	0	(?)+	Fresh dog hb.
15–43									
June 17	33.5	28	+	—	2,800	755	0	+	Crystalline dog hb., 7 gm.
15–34									
May 20	56.0	44	++++	0.25	4,000	1,360	0	(?)+	" " " 7 "
15–41									
June 17	36.5	48	++++	0.18	3,000	310	0	+	" " " 7 "
15–57									
June 16	24.5	48	++++	0.32	2,500	255	0	+	" " " 7 "
15–55									
June 16	40.5	46	++++	0.27	3,000	310	0	+	" " " 7 "

The preceding experiments and Table II show that the peritoneal cavity can transform hemoglobin into bile pigment with the same promptness as the pleural cavity. The two cavities are lined by a similar mesothelium, so there need be no surprise when the reactions are found to be similar. The difference in absorption from the two cavities is obvious. The pleural cavity gives slow absorption, most of the fluid introduced is recovered, and little if any of the formed bile pigment appears in the urine. The peritoneal cavity gives a pretty rapid absorption, most of the fluid introduced is taken up, and the bile pigments appear in the urine as a result.

DISCUSSION.

A difference in absorption from the pleural and peritoneal cavities has been noted and it comes out clearly in Tables I and II. The relatively rapid absorption from the peritoneal cavity causes the escape of bile pigments in the urine. Even in the pleural experiments, however, there is considerable absorption which indicates that in both pleural and peritoneal cavities there is an outflow of fluid. Whether there is interchange between the introduced fluid and the body fluids is uncertain, but much interchange is unlikely. In these experiments the dogs were all normal and their urine free from bile pigment at the beginning of the experiments, which means that the serum was negative to bile pigments by the test employed. That the blood pigments could have been absorbed into the blood, changed to bile pigments in association with the liver, and again diffused into the pleural fluid, is inconceivable. The observations in the pleural experiments (Table I) show that the urine was free from hemoglobin and bile pigments during the first forty-eight hours.

A theoretical objection could be raised to the experiments with the hemoglobin solutions in the peritoneal cavity. It could be argued that this solution comes in contact with the liver and could be modified in this manner. The solution does come in contact with the serous covering of the liver, but not with the hepatic epithelium. Diffusion through the serous epithelium of the liver is conceivable, but most improbable, and the pleural experiments are not open to such an objection.

The use of crystalline hemoglobin seems to cause more irritation of the serous surfaces than does a fresh solution of hemoglobin obtained from freshly laked red corpuscles. When this irritant action lasts over a period of three days there is undoubtedly some new formation of capillaries and proliferation of mesothelium, as well as an escape of various wandering cells. This reaction brings other extrahepatic factors into the equation and apparently accelerates the formation of bile pigment (Table I).

All this evidence indicates that the function of changing hemoglobin to bile pigment is not limited to any single cell or even to two types of cell. It may well be a function of endothelium and mesothelium as well as of hepatic epithelium. Perhaps wandering cells have this property of transforming blood to bile pigment. Many of these wandering cells probably have their origin from endothelium, and may they not retain this functional capacity? It is quite possible that other epithelium besides liver epithelium may be able to effect this transformation. More experiments are being carried out with this point in view. If we go a step further it may be suggested that living protoplasm in general can change hemoglobin to bile pigments.

Still other questions may be raised. Can tissue juices or ferments bring about this transformation of hemoglobin to bile pigment? We have performed many experiments with negative results, but have not yet given up the attempt although success seems unlikely.

CONCLUSIONS.

It is known that hemoglobin can be rapidly changed to bile pigment in a circulation confined to the head, neck, and thorax. This excludes direct liver participation (1).

These experiments show that hemoglobin can be changed to bile pigment within the pleural or peritoneal cavities.

This transformation can usually be detected after eight hours, and the amount can often be estimated quantitatively after an interval of twenty-four hours.

Such experiments demonstrate the importance which may attach to extrahepatic bile pigment formation. That bile pigments can be formed without direct liver activity is established beyond doubt.

It is highly probable that endothelium as well as mesothelium (serous cavities) can transform hemoglobin into bile pigment. It is possible that this property may reside in cell protoplasm in general.

BIBLIOGRAPHY.

1. Whipple, G. H., and Hooper, C. W., *Jour. Exper. Med.*, 1913, xvii, 612.
2. McNee, J. W., *Med. Klin.*, 1913, ix, 1125.
3. McNee, J. W., *Jour. Path. and Bacteriol.*, 1913–14, xviii, 325.
4. Virchow, R., *Virchows Arch. f. path. Anat.*, 1847, i, 379.
5. Guillain, G., and Troisier, J., *Rev. de. med.*, 1909, xxix, 465.
6. von der Bergh, A. A. H., and Snapper, J., *Berl. klin. Wchnschr.*, 1914, li, 1109.
7. Bradley, H. C., and Sansum, W. D., *Jour. Biol. Chem.*, 1914, xviii, 497.